CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

20-414

ADMINISTRATIVE DOCUMENTS

MEMORANDUM

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

DATE: February 5, 2003

FROM: Robert J. Temple, M.D.
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SUBJECT: Basis of approval of application under the "Animal Efficacy Rule," 21 CFR Part 314.600, Subpart I

TO: File NDA 20-414 – use of pyridostigmine bromide (PB) for prophylaxis against the lethal effects of Soman poisoning

Note: A previous version of this memorandum was filed in DFS on February 5, 2003. The present memorandum corrects a few minor typographical errors in the text. It also corrects typographical errors found in the table on page 7.

I.   Summary

Under the animal efficacy rule; 21 CFR 314.600-650, a drug may be approved for reducing or preventing life-threatening injury from various toxic substances on the basis of evidence of effectiveness derived from adequate and well-controlled studies in animals when it is ethically impossible to conduct controlled trials in humans and field studies after accidental exposure have been infeasible, and when those animal studies establish "that the drug product is reasonably likely to produce clinical benefit in humans."
21 CFR 314.610(a). Safety of the drug must also be established based on data in humans.

Reliance on animal studies to establish effectiveness is acceptable only when certain conditions are met: (1) there is a "reasonably well-understood" pathophysiology of the toxin and protectant, (2) effects have been demonstrated in more than one animal species expected to be predictive of the response in humans, (3) the animal studies show an effect on a clearly relevant endpoint related to the desired benefit in humans, such as enhanced survival, and (4) there is an ability to select an effective dose in humans based on PK, PD or other information in animals and humans.

Administration prior to exposure to Soman of pyridostigmine bromide (PB), with antidotes atropine and 2-PAM (pralidoxime), is clearly protective against Soman exposure in Rhesus monkeys and guinea pigs (the first part of requirement 2) for a relevant endpoint, survival (requirement 3). The monkey studies show very large effects, increasing the LD_{50} of Soman by more than 20 fold compared to atropine/2-PAM alone. This is sometimes described as having a "protective ratio" of 20, or 28-40 fold compared to no treatment, and these observations have been replicated in studies (two of which were inspected on-site) that have been found to meet GLP standards (see Rosloff review of July 24, 1996). Studies in guinea pigs also consistently show a substantial PR of 4-7 compared to no-treatment control (and 2-4 vs. atropine/2-PAM control); at least one large study has been presented in detail. There are also numerous other supportive studies in the literature in other species, conducted by many different investigators, although PR's are not as high. The available data allow selection of an effective dose in humans.

The reason there has been discussion and, to a degree, disagreement, about a) whether the expectations of the animal efficacy rule have been met, and b) the relevance of those data to humans are first, the apparent failure of some species (rats, mice, rabbits) to show substantial protection (i.e., a large protective ratio, the ratio of LD_{50} with PB to the LD_{50} without PB), and second, the lack of a clear and consistent quantitative relation of a potential surrogate measure of PB effect (inhibition of blood acetylcholinesterase, AChE) to protection. These concerns go to the question of whether we, in fact, do understand "reasonably well" the pathophysiology of the protective effect of PB (requirement 1) and whether the effect seen in monkeys and guinea pigs is in fact expected to be predictive of an effect in humans (second half of requirement 2). If we did not understand the pathophysiology it would be possible that it was the modest benefit in rats, mice, and rabbits (with absent effect in some studies) that is most relevant to humans, not the larger effect in monkeys and guinea pigs.

As I will describe below, there are good explanations, supported by considerable data, carried out by many investigators, of the apparent differences between animal species in their protective ratio response to PB (and their different sensitivity to Soman) that mitigate the concern arising from the apparent minimal protection in some species. These data show that it is defined differences among species that determine their sensitivity to Soman and the "protective ratio" of PB and that there is protection in all species studied. I therefore conclude, as does Dr. Katz, that the response of humans to PB is far more likely to be similar to that seen in monkeys and guinea pigs (a substantial protection from Soman lethality) than to the smaller protective ratio effect in rabbits and rodents. The data strongly support the proposed mechanism of action of PB in protecting against Soman toxicity (requirement 1) and lead me to conclude that the results in monkeys and guinea pigs will be predictive of the results in humans (requirement 2). As will be discussed further below, the apparent difference in protective ratios between monkeys/guinea pigs and rodents/rabbits does not appear to reflect a true difference in PB effect when one considers protection against specific amounts of Soman; the apparent difference can be explained by different levels of a Soman-binding enzyme, carboxylesterase, in different species that protects these species (rodents in particular) from Soman poisoning.

In what follows, I will rely in many cases on the more detailed discussions of many points by Dr. Rosloff in his memorandum filed on January 2, 2003 and updated January 14, 2003, as well as his original reviews dated July 24, 1996 and October 28, 1996, and by Dr. Katz in his recommendation for approval of PB dated January 20, 2003 and his addendum dated February 5, 2003.

II.   The proposed mechanism of PB protection

Soman is an irreversible inhibitor of acetylcholinesterase (AChE), which is widely present in the body and breaks down acetylcholine (ACh). Apart from its central (within the brain) effects and smooth muscle peripheral effects, which are treated primarily by atropine, Soman inactivates AChE in the neuromuscular junction (ACh is the critical mediator of nerve-stimulated muscle function), leaving the muscle in a chronically stimulated state (because ACh is not broken down) which quickly results in paralysis. This is similar to the way succinylcholine (which cannot be broken down by AChE) works to cause paralysis during surgery.

Although Soman's effect is irreversible, it can be removed from AChE by pralidoxime (2-PAM), which is always given with atropine after Soman exposure, if the AChE has not "aged" (become irreversibly inactivated). Soman, unfortunately, ages AChE very rapidly (in minutes) so that 2-PAM by itself has very little effect on Soman poisoning. It is much more effective against nerve agents like Sarin, which age much more slowly, leaving AChE that can be restored by the 2-PAM. Given Soman's rapid aging, 2-PAM will be useful in Soman poisoning only if it is given almost immediately after Soman exposure or if it is present when AChE that has been protected from Soman by PB (see below) becomes available again. If newly freed AChE were exposed to residual Soman without 2-PAM, it too would be inactivated by the Soman.

Pyridostigmine bromide (PB) is a reversible inhibitor of AChE. Indeed, it is used to treat myasthenia gravis, a disease of too few functioning ACh receptors and resulting muscle weakness; inhibition of AChE by PB allows ACh to remain present longer and thus improve muscle strength [too much PB, resulting in excessive ACh, can result in the same paralysis that succinylcholine causes, a so-called "cholinergic crisis," that is very similar to the muscle effects of Soman].

The role of PB in Soman poisoning has no relation to its pharmacologic (anticholinesterase) activity but rather to its ability to bind, reversibly, to AChE. When PB is present in the body before Soman exposure (it is not to be taken during or after Soman exposure), it "protects" some of the AChE from inactivation by Soman. When the Soman is eliminated, or probably even if it is still present but 2-PAM has been given to restore unaged AChE, and the PB is excreted, it yields free AChE that can sustain muscle function. Reactivation of the PB-affected AChE requires removal of the carbamate that PB added (decarbamoylation). It has been argued [Ellin and Kaminskis. J Pharm Pharmacol. 1989;41:633-635] that this process is faster in monkeys>guinea pigs>rabbits>rat, roughly corresponding to the size of the effect of PB in those species, which could explain part of the greater effect of PB in monkeys and guinea pigs.

The animal efficacy rule requires that the pathophysiological mechanism of the toxin-protectant relationship be "reasonably well-understood," which is not the same as knowing the relationship with certainty. As I will illustrate, there is a great deal of support, from many sources, for the proposed mechanism, and I therefore conclude that the pathophysiological mechanism is reasonably well-understood. It is particularly encouraging that the support explains both why PB works well, when it does, and why it doesn't seem to work as well (based on protective ratio) in some cases.

III.   The Animal Data on Protection against Soman

I will not summarize all the individual studies, but the following table shows the protective effect of PB in various species, usually by comparing the LD_{50} of Soman with and without PB in animals given atropine and 2-PAM after exposure. The numbers are from Dr. Rosloff's review of submitted data and various identified publications. Note that there are other supportive studies that do not calculate change in LD_{50}, but that show protection against multiples of the established lethal doses.

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Table 1

Species	LD$_{50}$ (µg/kg)				Δ LD$_{50}$ (µg/kg) and PR vs. control ( )			Δ LD$_{50}$ (µg/kg) and PR vs. At/PAM	Source
	Control	At/PAM	At/PB	At/PAM/PB	At/PAM	At/PB	At/PAM/PB		
Rhesus	13.0		176			163 (13.5)		---	Rosloff 1/9/03 (Dirnhuber)
*Rhesus	15.3	25.1		>617	9.8 (1.6)	---	>602 (>40)	>592 (24.6)	Rosloff 1/9/03 (Joiner, 1988)
*Rhesus	6.5	8.8		182	2.3 (1.4)		176 (28)	173 (20.7)	Rosloff 7/24/96 NDA Vol 2.10
*Guinea Pig	26.6	54.0		190	27.4 (2.0)	---	163.4 (7.1)	136 (3.5)	Rosloff 1/9/03 (Lennox)
*Guinea Pig	30	54		135	24 (1.8)		105 (4.5)	81 (2.5)	Rosloff 7/24/96 Capacio 93
*Guinea Pig	30	102		192	72 (3.4)		162 (6.4)	90 (1.9)	Rosloff 7/24/96 Jones 85
*Guinea Pig					(1.7)		(6.8)	(4.0)	Rosloff 7/24/96 Inns & Leadbetter 83
Guinea Pig	28		146		---	118 (5.2)	---	---	Rosloff 1/9/03 (Maxwell)
*, ** Rabbit	15.2	28.4		42	13.2 (1.9)	---	26.8 (2.8)	13.6 (1.5)	Rosloff 1/9/03 (Koplovitz 92)
Rabbit	20		121		---	101 (6.1)	---	---	Rosloff 1/9/03 (Maxwell 88)
**Rabbit	15.4		36.5	41.5	---	21.1 (2.4)	26.1 (2.7)	---	Rosloff 1/9/03 (Joiner 89)
*,** Rabbit	15.4	20.9		22.5	5.5 (1.4)		7.1 (1.5)	1.6 (1.1)	Rosloff 1/9/03 Joiner 89
*,** Rabbit	11.5	25.3		35.7	13.8 (2.2)		24.2 (3.1)	10.4 (1.4)	Rosloff 1/9/03 Sultan and Lennox 83
*, ***Rat	157	199		374	42 (1.3)	---	217 (2.4)	175 (1.9)	Rosloff 1/9/03 (Lennox)
Rat	126		262		---	136 (2.1)	---	---	Rosloff 1/9/03 (Maxwell 88)
*Rat	61.7	76.6		87.1	14.9 (1.2)		25.4 (1.4)	10.5 (1.1)	Rosloff 1/9/03 Anderson 92
*,** Mouse	90.7	159.0		204.4	68.3 (1.8)	---	113.7 (2.3)	45.4 (1.3)	Rosloff 1/9/03 (Koplovitz 84)
*,** Mouse	107.4	186.2		238.9	78.8 (1.7)	---	131.5 (2.2)	52.7 (1.3)	Rosloff 1/9/03 (Koplovitz 84)
*, **Mouse	102	112		275	10 (1.1)		173 (2.7)	163 (2.5)	Rosloff 1/9/03 Sultan & Lennox 83

*Has comparison of At/Pam/PB vs. At/PAM, i.e., the optimal control
**Arguably too little atropine
***Mecamylamine instead of 2-PAM

A few things are clear.

1. Animals differ considerably in their sensitivity to Soman, with monkeys, guinea pigs (GP), and rabbits having LD_{50}'s in the 10-30 μg/kg range, and rats and mice in the 100-150 μg/kg range.

2. Studies vary in design. The best-controlled studies compare the LD_{50} on PB, when followed by the atropine and 2-PAM that protect against non-skeletal muscular effects of Soman (the atropine) and restore unaged AChE (the 2-PAM), with the LD_{50} on atropine and 2-PAM alone. These studies clearly isolate the contribution of PB. A less ideal measure, based on the known relatively small effect of atropine alone, is to compare the LD_{50} on PB and atropine with the LD_{50} on no treatment, but this design gives informative data only if the effect is clearly larger than that of atropine (At) alone. "Protective ratios" (PR), i.e., the LD_{50} on PB ÷ LD_{50} control, differ considerably among species. Looking at the most appropriate measure, which is LD_{50} At/PAM/PB ÷ LD_{50} At/PAM, the ratio is 20-24 for monkeys [it was 13 in a study comparing At/PB with no treatment where the effect of atropine alone would not be nearly so large]; 1.9-4 for guinea pigs in various studies, and consistently less than 2 in other species. A comparison of At/PB vs. no treatment in the guinea pig gave a PR of 5.2, larger than At/PAM alone in any study; At/PAM (presumably a greater effect than atropine alone) never gave a PR greater than 2, except for one study in the guinea pig (3.4, Jones 85) and one in the rabbit (2.2, Sultan and Lennox 83) and was <2 in all other studies (some of which may have used too little atropine). While it would be difficult to prove this rigorously, adequate atropine may be necessary for PB to show its protective effect. If atropine is not there to protect against central and atropine-responsive smooth muscle effects, PB may not be able to show its effect on the peripheral (neuromuscular junction) Soman toxicity. The studies in Table 1 that had relatively low atropine doses are marked with a double-asterisk. All but one showed little effect of PB.

    It is these interspecies differences that lead to questions about what the mechanism of effect really is.

The effect of PB in various species, while quite different with respect to PR's, is not as different with respect to the actual change in LD_{50} dose, except that monkeys seem to be particularly well-protected by PB and the rabbit seems to show little effect (although all the rabbit studies except Maxwell 88, which did show an effect, used relatively little atropine). Thus (with the Joiner monkey study as an outlier), several species show fairly consistent increases in μg/kg LD_{50} with At/PAM/PB vs. At/PAM alone, including the monkey (173 μg/kg, 2nd NDA study), GP (81-136 μg/kg) and the rat (175 μg/kg in one study but only 10 μg/kg in another); the mouse shows an effect of PB of about 50 μg/kg in two studies, but this may be because too little atropine was administered although a third study showed a larger effect, 163 μg/kg, despite use of the same dose of atropine. In the less optimally controlled studies, i.e., those comparing At/PB vs. no treatment (presumably representing some overestimate of the PB effect) there also were similar results including 163 μg/kg for monkeys (Dirnhuber), 101 μg/kg for rabbits (Maxwell 88), 118 μg/kg for guinea pigs (Maxwell 88) and 136 μg/kg in the rat (Maxwell 88). Although these last studies overestimate the PB effect, the effects are considerably larger than atropine/PAM effects in the same species (in other studies).

There is obviously considerable variability in these data, and some studies show no real impact of PB at all [mainly in the rabbit (Koplovitz) and mouse, where inadequate atropine is said to have been used]. Dr. Rosloff (1/9/03) points out, however, that there appear to be a few studies with adequate atropine in which there was little evidence of protection (e.g., the Anderson 92 rat study).

I believe some variability is not wholly surprising, however, as LD_{50}'s are never precise without studies of considerable size. Despite the variability, there is a fairly consistent 80-175 µg/kg increase in LD_{50} from PB/At/2-PAM compared to At/2-PAM alone (rabbits being the main exception, again perhaps because too little atropine was given) even if the PR's are very different. An explanation of how this might occur follows.

IV.   Role of endogenous or exogenous esterases

It has been postulated that the differences in Soman sensitivity (10-fold among species) and the differences in protective ratios can be explained by the variable presence in different species of a circulating enzyme, carboxylesterase (CaE) that binds Soman in the bloodstream and inactivates it, and that the less responsive species in terms of PR are those with high levels of CaE.

Dr. Rosloff explains how this could happen:

Suppose that CaE can bind (inactivate) Soman and that its presence is variable among species (considerable data, including a recently completed Army study, document this variability). Also suppose that the effect of PB on the toxicity of free Soman in blood is the same in all species. Consider 2 species, one (monkey) with a level of CaE of zero, another (rat) with a high CaE, and suppose that the LD_{50} blood level of unbound Soman is the same in all species, even though the LD_{50} dose is variable because not all of the Soman given is active.

In the monkey, with no CaE, the LD_{50} dose of 10 µg/kg leads to an LD_{50} Soman concentration (blood level) of, say, 1 µg/ml. This LD_{50} Soman blood level is raised to 10 µg/ml by PB and, assuming dose-proportional absorption, the dose of Soman needed to attain that blood level is 100 µg/kg, giving an LD_{50} protection of 90 µg/kg and a PR of 10. Now suppose the rat, which has CaE to bind Soman, has to be given a Soman dose of 100 µg/kg to achieve the actual LD_{50} dose of 10 µg/kg because 90 µg/kg of Soman is bound to circulating CaE. This leaves an "actual" Soman dose of 10 µg/kg (100-90 µg/kg), again leading to the LD_{50} concentration of 1 µg/ml.

If PB has the same effect in the rat on the LD_{50} Soman concentration, then PB will raise the Soman LD_{50} concentration to 10 µg/ml (same as monkey). To achieve this, however, one needs to give 100 µg/ml (90 µg/kg of which is used to saturate CaE, resulting in an actual free Soman dose of 10 µg/kg) plus 90 µg/kg more, to increase the blood level to the LD_{50} of 10 µg/ml. The actual LD_{50} increase in Soman dose caused by PB is 90 µg/kg in both species but the calculated PR's are 10 (100 + 10) in the monkey and 1.9 (190 + 100) in the rat. This is shown in the following table.

	PB Untreated		PB Treated	
—	LD_{50} Soman Dose	LD_{50} Soman Concentration (free)	LD_{50} Soman Dose	LD_{50} Soman Concentration
Monkey (low CaE)	10 µg/kg	1 µg/ml	100 µg/kg	10 µg/ml
Rat (high CaE)	100 µg/kg	1 µg/ml	190 µg/kg	10 µg/ml

PR monkey = 10
PR rat = 1.9

This explanation is plausible, although dependent on critical assumptions, notably the existence of a saturable CaE "sink" to hold and inactivate the Soman, and would be speculative, but for two further lines of evidence:

1.   .   Increased sensitivity to Soman and increased response to PB in "PB-insensitive" (based on PR) species treated to inhibit their CaE activity, fully testing the mechanism described above.

2.   Decreased sensitivity to Soman by infusion of exogenous esterases to previously sensitive species showing the role of a Soman-binding "sink."

I discuss these two further lines of evidence more fully below.

1.   Inhibition of CaE activity causes decreased LD_{50} and increased PR response to PB in "PB-insensitive" (based on PR) species.

There is clear evidence that the Soman LD_{50} is related to the level of CaE. Maxwell, Lanclos, and Benschop show a linear relation of LD_{50} to CaE, as shown (read from figure in the publication) below. [Note: there are also more recent data from the Army, described below.]

Species	LD_{50} (µg/kg)	CaE (µM)
monkey	15	0
rabbit	22	0.5
guinea pig	35	1.0
rat	100	4.5
. mouse	120	6.2

The level of CaE can be reduced; when this is done, the LD_{50} changes as the table above would predict. Moreover, when CaE is reduced the response to PB changes as the model described above predicts. Maxwell, et.al. [Effect of CaE inhibition on carbamate protection against Soman toxicity. J Pharmacol Exp Therap 1988; 246:986-991; Comparison of antidote protection against Soman by pridostigmine HI-6, and acetylcholinesterase. J Pharmacol Exp Therap 1993; 264:1085-9] used an inhibitor of CaE, CBDP, to pre-treat rats, mice, guinea pigs and rabbits and observed the effect of this treatment on PB (as well as physostigmine, a related reversible anticholinesterase) protective ratios. If the model described above is correct, all of the animals now with much greater sensitivity to Soman (i.e., lower LD_{50}) should show a larger PR with PB treatment. All of these studies all compared controls given no treatment with animals given both atropine and PB, not the best design. A preferable study design would have given control animals atropine and 2-PAM, and given treated animals all 3 drugs. It is possible that CBDP would enhance the PB effect of any protectant, including atropine, by reducing the CaE "sink," but there is evidence that the effect of atropine alone is small compared to PB with atropine even in species with little or no CaE, and Dr. Rosloff cites a paper [Maxwell and Brecht. The role of carboxylesterase in species variation of oxime protection against Soman. Neuroscience and Biobehavioral Reviews. 1991; 15:135-139] showing that the protective effect of atropine was not potentiated by CBDP. [Another paper, also cited by Dr. Rosloff, shows potentiation, but at a much higher dose of CBDP.]

The results of the CBDP studies are shown in Table 2. The PR's and protection difference in the absence of CBDP are the same as those shown in Table 1 (except for the Maxwell mouse data), and are now restated in the left side of Table 2, and the results of

CBDP treatment are shown on the right side of Table 2. The results are striking. The 4 species, now with CaE substantially removed from blood and lung (though not from liver) by CBDP, have very similar sensitivity to Soman and also show similar effects of PB on LD_{50} in μg/kg (and they remain similar to monkey for this measurement). But the PR's are now greatly increased by PB, not quite as high as in some monkey studies but similar to Dirnhuber's similarly designed study (Table 1), just as the model described above would predict if the CaE were removed, thereby making the species "monkey-like" in their CaE levels. [Note that Maxwell showed that CBDP did not at all affect AChE, an early concern of Dr. Rosloff.] Soman decreased both AChE and CaE activity.

Recent data from the U.S. Army confirm the published evidence related to CaE levels in various species:

Species	CaE level (micro M)
Rat	1.95
Guinea Pig	0.068
Rhesus	0.027
Humans	-0.022

Table 2

	No CBDP				CBDP			
	LD$_{50}$ (µg/kg)				LD$_{50}$ (µg/kg)			
	Control	PB	PR	Protection	Control	PB	PR	Protection
Rat	126	262	2.1	136	15.6	133	8.5	117
GP	28	146	5.2	118	12.3	123	10.0	111
Rabbit	20	121	6.1	101	9.8	112	11.4	102
Mouse					22	213	9.7	191
	physostigmine similar in rat, GP, rabbit				physostigmine similar in rat, GP, rabbit			

2.   Infusion of exogenous esterases

If it is true that the endogenous CaE "sink" explains resistance to Soman, and that removal of the endogenous esterase makes animals sensitive to Soman, it should be possible to make sensitive animals, like monkeys, resistant to Soman by administering an esterase prior to Soman exposure.

Maxwell and co-workers, and others, have explored the ability of esterases, such as human, equine, or fetal bovine butyrylcholinesterase, to protect sensitive species, including Rhesus monkeys and guinea pigs. These studies did not yield a calculated increase of LD_{50}; rather they showed that animals could survive and tolerate multiples of the Soman LD_{50} without symptoms of organophosphate poisoning. Two typical studies are the following:

a.   Broomfield and co-workers [Protection by butyrylcholinesterase against organophosphorus poison in non-human primates. J. Pharmacol and Exper Therap 1991; 259:633-638.] showed that Rhesus monkeys given 503 nmoles of BuChE i.v. tolerated 2-4 LD_{50}s of Soman without any mortality.

b.   Allen and co-workers [Prophylaxis against Soman inhalation toxicity in guinea pigs by pretreatment alone with human butyrylcholinesterase. Toxicology Sci 1998; 43:121-128] studied the effects of human butyrylcholinesterase on the mortality and toxicity of Soman in guinea pigs, finding that the animals could tolerate exposures of about 5 times the LD_{50}, depending on the butyrylcholinesterase dose.

The results of studies with CBDP and exogenous esterases, together with the monkey and guinea pig data, strongly support our understanding of the pathophysiology of Soman and PB protection (requirement 1 of the animal efficacy rule) and lead to an expectation that the effect in animals will predict a similar effect in humans (requirement 2).

V.   Modeling

A report by Dr. Lee, dated January 3, 2003, brings together the relationship of CaE, the effects of CBDP, the dose of Soman, and the protective effect and protective ratios of PB, describing these relationships in a single model that is consistent with the actual available data. It shows:

1.   A reasonably linear relationship of LD_{50} to plasma CaE and an increased LD_{50}, but still with a linear relationship of LD_{50} to CaE, when there is 70% inhibition of circulating (RBC) AChE by PB.

2.   When CaE is naturally absent (monkeys, marmosets) or reduced to near absence by the administration of CBDP (rat, guinea pig, rabbit, mouse), the LD_{50} is small (10-20 µg/kg or so), as described above, but it is larger (100 µg/kg) when the endogenous level of CaE is higher (mouse, rat), and it is increased by about 100 µg/kg by PB plus atropine, with the final value depending on plasma CaE. Thus, PB plus atropine raises LD_{50} in the no-CaE state by about 100 µg/kg, to about 120 µg/kg, and in the high-CaE state by about 120 µg/kg, to about 250 µg/kg. The exception seems to be the monkey, where PB has a very large effect.

VI.   Conclusions about the understanding of the pathophysiology of the toxin-protectant relationship and whether the effect observed in animals is expected to predict the human response

Like Dr. Katz in his January 20, 2003 memorandum, I find the totality and consistency of evidence convincing with respect to the mechanism of PB protection against Soman toxicity. The difference in both sensitivity to Soman and response to PB is explained by differences in CaE levels and the fact that insensitive animals can be made sensitive to Soman and responsive to PB by removing CaE with CBDP supports this explanation. This explanation is supported by the individual studies described above and by Dr. Lee's modeling efforts. Although it is true, as Dr. Rosloff points out, that not every study supports this model, biological and interstudy variability is to be expected, especially when the number of animals in each study is small, and the overall weight of evidence is great. We therefore do have a reasonably well understood pathophysiological mechanism of the toxicity of Soman and the protective effect of PB. The consistency of PB's effect in animals with low levels of CaE (whether the low level occurs naturally or is induced by CBDP) leads me to conclude that the animal data are predictive of the human response. Thus the first three requirements of the animal efficacy rule are fulfilled.

VII.   Dose Selection

We know that human doses of pyridostigmine bromide 30 mg t.i.d. inhibit red blood cell cholinesterase by 20-40%, a level at or above what appears to protect monkeys from the lethal effects of Soman. Dr. Booth (OCPB) calculated that the AUC in humans after this dose was at or above the AUC in protected monkeys. The human exposure after this dose will thus be similar to the exposure that was effective. The 30 mg t.i.d. dose of PB thus appears to be an effective dose in humans based on the animal experience.

VIII.   Other Issues

1.   Overall Safety

The Division has reviewed available human safety data and Dr. Katz's memo of February 5, 2003 summarizes these evaluations. The long experience with much larger chronically administered doses of PB in myasthenia gravis is an important part of the human safety data base. The myasthenic patients should be susceptible to any central effects of PB, as their abnormality is in their skeletal muscle. There are also numerous studies conducted by the Army and Air Force that show no serious adverse effects of PB. There have also been several external reviews of a possible relationship of PB exposure to the "Gulf War Syndrome." These conclude that no such relationship is supported by available data, although in some of the reports there were suggestions for further study and criticisms of the available data. There have also been reviews by Dr. Rosloff of a variety of studies of PB interactions with various insecticides (DEET and permethrin); although there are suggestions of interactions, it is not clear that any of these results relate to symptoms that have been reported in humans.

2.   Drug-drug interactions in the PPI

The animal efficacy rule at 21 CFR 314.610(b)(3) requires that applicants prepare labeling to be provided to patient recipients. This information is to include, among other things, information regarding drug interactions. The Agency therefore considered including a section in the patient package insert reading as follows:

I have concluded that this information should not be included in the patient package insert because the product is being approved for military combat use only and we would not want people not to use it except for substantial concerns. The suggested information would be distracting under the circumstances, particularly given the very small dose (30 mg t.i.d.) used compared with that used in myasthenia gravis. Also, examination of current Mestinon labeling and Goodman and Gilman did not suggest a basis for avoiding PB in the situations described. (There is no clear interaction with propranolol or other beta blockers and potential users will not be users of treatments of dementia, especially at the proposed low PB doses.) Labeling for mefloquine includes no warning information related to PB or similar drugs, but it is possible that both drugs increase heart rate modestly.

IX.   Conclusion

For reasons given above, I conclude that the application for PB for use as prophylaxis against the lethal effects of Soman poisoning should be approved under 21 CFR 314 Subpart I (the "animal efficacy rule"). The pathophysiology of the toxic effects of Soman and the protective effects of PB are reasonably well understood and the protective effect of PB, shown in monkeys, guinea pigs, rats, mice, and rabbits (shown in the last 3 species best when their endogenous carboxylesterase is inhibited), can be expected to predict the human response on an important measure, mortality. There is a reasonable basis for the human PB dose of 30 mg t.i.d. Considerable human data indicate that Soman can be given safely.

Robert Temple, M.D.

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/s/

Robert Temple
3/25/03 12:12:10 PM
MEDICAL OFFICER

MEMORANDUM

DATE: January 20, 2003

FROM: Russell Katz, M.D.
           Director
           Division of Neuropharmacological Drug Products/HFD-120

TO: File, NDA 20-414

SUBJECT: Recommendation for Action on NDA 20-414, for the use of
pyridostigmine bromide tablets as pre-treatment for soman poisoning

NDA 20-414, for the use of pyridostigmine bromide tablets as pre-treatment for
poisoning by soman, an organophosphate nerve agent, was submitted by the
Department of the Army on 3/4/94. A Not Approvable letter was issued on May
27, 1997, and a second Not Approvable letter was issued on September 18,
1998. A brief summary of the relevant issues is presented below.

Pyridostigmine is a reversible acetylcholinesterase inhibitor.
Acetylcholinesterase is the primary enzyme that catabolizes acetylcholine, the
neurotransmitter at the neuromuscular junction (NMJ), as well as at central
nervous system (CNS) sites and other peripheral muscarinic receptors. Soman
is an organophosphate nerve agent that binds irreversibly to cholinesterase.
This irreversible binding ultimately results in respiratory failure from the
accumulation of acetylcholine 1) at the NMJ of the muscles of respiration, 2) at
muscarinic receptors in secretory glands and smooth muscle of the respiratory
tree, resulting in bronchoconstriction, and 3) at receptors in the brain, resulting in
central depression of respiration. The sponsor proposes that pyridostigmine
protects against the peripheral respiratory failure (pyridostigmine does not enter
the CNS appreciably) if it is bound (reversibly) to the cholinesterase at the time of
exposure to soman. In conjunction with atropine (an anti-cholinergic agent at
muscarinic receptors) and 2-PAM (an agent which has the capacity to cleave the
soman off of the cholinesterase before the binding becomes irreversible), the
pyridostigmine is considered to "protect" the enzyme from irreversible inhibition
by soman. When soman is cleared, pyridostigmine-induced inhibition is
reversed, releasing enzyme that is then capable of functioning normally at
peripheral sites. Of course, in order for the treatment to be effective, the timing
of these events must be appropriate (for example, presumably pyridostigmine
should dissociate from the enzyme as the level of soman is reducing, etc.).

The Army had proposed that the application be approved under Subpart H of the
NDA regulations. These regulations permit the approval of a drug product on the
basis of adequate and well-controlled clinical trials that demonstrate that the drug
has a beneficial effect on a surrogate marker reasonably likely to predict the
clinical benefit of interest; that is, on the basis of an effect on an unvalidated

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surrogate marker. Clearly, adequate and well-controlled clinical trials demonstrating the direct clinical benefit of the treatment in patients exposed to lethal nerve agents cannot be conducted ethically; for this reason, the provisions of Subpart H were considered applicable.

The surrogate marker proposed by the Army was the drug's effect on RBC cholinesterase. In the Army's view, an approximate 20-30% inhibition of RBC cholinesterase in humans would be associated with effectiveness. This conclusion was based on studies performed in animals; in particular, on studies performed in the rhesus monkey, and, to a lesser degree, on studies performed in the guinea pig. These studies demonstrated a marked increase in the Protective Ratio or PR (the ratio of the Soman LD50 with pyridostigmine pre-treatment to the Soman LD50 without pre-treatment) in both species (monkeys>guinea pig). The Army's approach, which the Agency agreed was appropriate, was to "validate" the surrogate in animals, thereby "establishing" that the effect on the surrogate predicted the desired clinical benefit (in animals), and then develop a dosing regimen in humans that would achieve the desired effect on the surrogate (in this case, RBC cholinesterase inhibition).

Unfortunately, a definitive study in the monkey demonstrated essentially complete protection (survival) at doses of pyridostigmine that resulted in a degree of RBC cholinesterase inhibition equal to that of the control; that is, there was no correlation between the degree of RBC cholinesterase inhibition and the desired clinical benefit. While correlation of the surrogate and the clinical benefit would not constitute true validation, it is at the very least a necessary step in the validation process. Therefore, the lack of such a correlation ruled out the proposed surrogate's utility as a predictor of effectiveness in monkeys, as well as, of course, in humans.

Further, the 9/18/98 Not Approvable letter noted that there were inconsistencies in the responses of various animal species to pyridostigmine pre-treatment. Specifically, while monkeys, and to a lesser extent, guinea pigs, responded to pre-treatment (with increased PRs in the face of exposure to soman), rats, rabbits, and mice did not, even in the face of considerable degrees of RBC cholinesterase inhibition. This was considered important because extrapolating from evidence in animals to humans would require, at least, a detailed understanding of the mechanism of action of pyridostigmine's effectiveness. A lack of effectiveness in some species would cast doubt on the understanding of this mechanism (the events at the NMJ in all species studied being essentially the same), and, therefore, would make extrapolation to humans even more problematic than it might otherwise be.

Because of the failure to validate the surrogate, the Agency proposed that the Army pursue the "validation" (in animals) of a different surrogate, a surrogate presumed to be more reflective of the presumed mechanism of action of pyridostigmine (that is, it was believed that the original surrogate might not have

been validated for many reasons, even if the treatment was effective) . This new surrogate would be the degree of decrease in soman-induced cholinesterase inhibition produced by pyridostigmine pre-treatment. It was anticipated that this approach would require a number of studies, including a study in humans treated with pyridostigmine whose RBC's would be exposed, ex vivo, to soman. The degree of the resultant decrease in RBC cholinesterase inhibition achieved in humans could be correlated with similar measurements in various animal species (exposed in vivo to soman) in which this surrogate could be correlated with survival as well as with function at the neuromuscular junction. If the appropriate correlations could be made, such a program might have been considered strong support for the use of the new surrogate, and, therefore, the presumed effectiveness of the treatment in humans.

In addition, the Army offered an explanation for the apparent failure of pyridostigmine to provide protection against soman-induced mortality in rats, rabbits, and mice.

Specifically, the sponsor proposed that the relative resistance to pyridostigmine pre-treatment in these latter species is related to significant levels of carboxylesterase (CaE), an enzyme that binds, and therefore effectively inactivates, soman (this action of CaE is well accepted). Presumably, in these species, the carboxylesterase rapidly inactivates the soman, resulting in relatively high LD50s (without pyridostigmine). The addition of pyridostigmine (as pre-treatment) produces only a.minor effect, therefore, as measured by the PR. However, the sponsor argued that there was a similar **absolute increase** in the LD50 of soman in all species with pyridostigmine pre-treatment, suggesting that it is, in fact, effective in all species. Finally, the sponsor noted that monkeys have very low levels of carboxylesterase, as do humans, and that, therefore, humans would be expected to respond similarly to monkeys, the species in which pyridostigmine has a clear effect on mortality.

Subsequent to the 9/18/98 Not Approvable letter, the Army and the division engaged in numerous discussions about the specifics of a development program designed to validate the new proposed surrogate discussed above.

However, in the interim, the Agency, on 5/31/02, adopted new regulations governing the approval of drugs when human efficacy studies are not ethical or feasible. These new regulations, under Subpart I of 21 CFR 314, (so-called "animal rule"), apply to:

> ...new drug products that have been studied for their safety and efficacy in ameliorating or preventing serious or life-threatening conditions caused by exposure to lethal or permanently disabling toxic biological, chemical, radiological, or nuclear substances.

These new provisions are to be applied when definitive human efficacy studies are unethical, and only in cases in which other regulatory mechanisms (e.g., Subpart H) are not available. Although it is true that considerable effort has gone into attempting to identify, and "validate" in animals, a surrogate marker on the basis of which approval could be granted (approval under Subpart H), it is fair to say that, at this time, there is clearly no accepted surrogate marker that could serve this purpose. Discussions I have had with Dr. Robert Temple, Director of the Office of Medical Policy, suggest that Subpart I may be invoked if there is no immediately available, well accepted surrogate, even though attempts may be underway to develop such a surrogate in the future. With this potential impediment to its use removed, then, Subpart I appears to be an appropriate mechanism under which to consider the approval of pyridostigmine.

Specifically, the rule permits the approval of a treatment on the basis of "...adequate and well-controlled animal studies when the results of those animal studies establish that the drug product is reasonably likely to produce clinical benefit in humans."

The rule further states that the Agency will rely on such studies only when:

1) the pathophysiologic mechanism of the toxic substance and the mechanism of the proposed treatment are "reasonably well-understood",
2) the benefit of the treatment is established in more than one species expected to predict the response in humans, unless the treatment is effective in a single species that is "...a sufficiently well-characterized animal model for predicting the response in humans;",
3) the endpoint in the animal studies is clearly related to the expected benefit (generally survival or prevention of important morbidity) in humans, and,
4) the data allow the selection of an effective dose in humans.

Approval under this rule is subject to three additional requirements:

1) a post-marketing study to verify the drug's clinical benefit must be performed when these studies are feasible and can be done ethically,
2) the distribution of the drug can be restricted to insure its safe use, if necessary, and,
3) the sponsor must draft and distribute to patients information that explains that the approval is based on studies in animals, as well as other information that will permit the drug to be used safely.

The rule, therefore, is intended to permit approval on the basis of the results of studies in animals when it can be concluded that the animal studies are reasonably predictive of human effectiveness. Such a mechanism differs from the typical approval (which, of course, is based on adequate and well-controlled trials in humans with a naturally occurring disease) in numerous important ways (besides the obvious reliance on animal data): the rule requires that the

mechanism of action of the drug be reasonably well understood, and that the mechanism of the pathophysiology of the illness (in this case, the mechanism of the toxicity of soman) also be well understood. It is clear that extrapolation from animals to humans must, at least, depend upon these criteria; it is only in this case that we can hope to be able to conclude that the drug will be reasonably likely to be effective in humans. It is therefore well accepted that the use of this rule will require many more assumptions than are currently made in drug approval (for example, for a typical drug product, approval is based upon an empirical finding of benefit on a valid measure of patient functioning; this does not imply, or require, an understanding of the relevant mechanism(s) of action of the drug, or a detailed understanding of the pathophysiology of the disease being treated). Even if these actions and elements are well-understood, approval under Subpart I will always involve a major assumption; namely, that data in animals predict human response.

As a result of the existence of Subpart I, and its apparent applicability to this case, we had several discussions with the sponsor about re-submitting the NDA with a proposal for consideration under these rules.

The Army re-submitted the application on 1/3/03. This application (and related data, primarily from the archival literature and previous submissions by the sponsor), has been reviewed by Dr. Barry Rosloff, Pharmacology Team Leader (review dated 1/14/03), Dr. Kevin Prohaska, medical reviewer (reviews dated 1/7/03 and 1/9/03), Dr. Brian Booth, Office of Clinical Pharmacology and Biopharmaceutics (review dated 1/8/03), Dr. Ronald Kavanagh, Office of Clinical Pharmacology and Biopharmaceutics (review dated 1/8/03), Dr. Janusz Rzeszotarski, chemist, Dr. Karen Lechter, Division of Surveillance, Research, and Communication Support (reviews dated 12/31/02 and 1/10/03), and Dr. Lisa Stockbridge, Division of Drug Marketing and Advertising and Communications (review dated 1/10/03).

The primary goal of the sponsor is to establish that the elements of Subpart I have been met. In the rest of this memo, I will describe the data and arguments offered by the sponsor to support this conclusion, and offer the Division's recommendation for action on the application.

Mechanism of Soman toxicity and pyridostigmine's action as pre-treatment

As Dr. Rosloff notes, the mechanism of soman-induced injury is generally considered to be the result of irreversible inhibition of acetylcholinesterase at the neuromuscular junction (as well as at other critical sites, described above), with resultant persistent stimulation of cholinergic receptors, leading ultimately to respiratory depression (as well as perhaps other critical dysfunction) and death.

Pyridostigmine pre-treatment, in combination with treatment with atropine and 2-PAM, is clearly effective in monkeys, and, to a lesser extent, guinea pigs in

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decreasing the mortality of soman. This is a fact, but the mechanism of this protection cannot be said to be known with assurance, or even that it is obviously "reasonably well-understood", the standard stated in the rule. The presumed (and, it must be stated, generally accepted) mechanism, as described earlier, is that pyridostigmine reversibly binds to cholinesterase, thereby protecting the enzyme from irreversibly binding with soman. Ultimately, soman is cleared (and to some extent cleaved off the enzyme before the binding becomes irreversible by 2-PAM), the pyridostigmine-induced inhibition is reversed, and sufficient cholinesterase becomes available to support functioning.

Unfortunately, attempts to correlate cholinesterase inhibition with survival, as noted above, have failed. Such a correlation would not, of course, firmly establish the proposed mechanism, but it would provide considerable support for it. Of course, it may be that the proposed mechanism is absolutely correct, but that RBC cholinesterase inhibition is an inappropriate marker for the degree of cholinesterase inhibition at the anatomic sites presumed necessary for survival (e.g., the NMJ). As described earlier, the Army is in the process of examining a surrogate that may be more relevant (that is, the degree of pyridostigmine-induced protection from soman-induced injury). However, these studies have not been done.

Pyridostigmine, like most drugs, has multiple pharmacologic actions, some of which may be unknown at this time. Therefore, it is possible that its effectiveness as a pre-treatment (in certain species) may be due to multiple mechanisms. Further, even though the mechanism of soman-induced injury is considered to be well-understood, this does not establish (or perhaps even suggest) that the only way in which pyridostigmine protects sensitive species is by "protecting" the cholinesterase, as presumed. A pre-treatment could affect any number of steps in a pathophysiologic pathway set in motion by another agent which irreversibly binds to the enzyme. So, understanding the initiating events of soman-induced death in no way implies a mechanism of action of a treatment that prevents that death.

On the other hand, however, it must be acknowledged that pyridostigmine is a drug about which there exists an enormously rich and voluminous literature, and its ability to reversibly bind to cholinesterase is well documented. Further, and critically, no other important action of the drug is as well documented and accepted as this. While, again, not constituting proof, this does at least suggest that, in those species which respond to pyridostigmine, it might be reasonable to conclude that the mechanism of action of pyridostigmine as a pre-treatment, in the presence of atropine and 2-PAM, is as proposed.

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Effectiveness in Multiple Species

The rule further requires that the treatment be effective in multiple species, or, if not, it should be effective in a species which is a sufficiently well-characterized model of the condition in humans.

On face, two observations seem clear. First, the treatment appears not to be effective, or certainly not equally effective, in all species tested, at least as assessed by the PR (defined above; in the "non-sensitive" species, the PR is about 2, compared to a PR in the monkey of 20->25, and in the guinea pig about 5-6). While the rule does not require that the treatment be effective in all species tested, the absence of an (apparent) effect in one or multiple species, without an adequate explanation for why these species are not relevant to humans, does appear to cast doubt on the Army's assertion that the mechanism of effectiveness is sufficiently well-understood to permit a conclusion that the effectiveness in the sensitive species predicts human responsiveness to the treatment.

Second, it is not immediately obvious that the species in which pyridostigmine is effective are better models of human responsiveness than the species in which it appears not to be effective.

As noted above, however, the sponsor addresses both of these points.

First, they argue that the PR is not the appropriate metric with which to evaluate the ability of pyridostigmine to protect, but that the **absolute increase** in the soman LD50 is more appropriate (that is, the difference between the soman LD50 with pyridostigmine minus the soman LD50 without pyridostigmine). The sponsor argues that this increase is relatively constant across species, suggesting that the pre-treatment is, measured in this way, effective in all species.

Their second point is further relevant to this argument.

As noted above, the sponsor argues that the differences seen among species is directly related to the activity of carboxylesterase (CaE), an enzyme that rapidly inactivates soman. Species with high levels of CaE provide, as Dr. Rosloff describes, a "sink" that effectively de-activates an initial "dose" of soman, resulting in relatively high initial LD50's of soman, which obscure the additional protective effect of pyridostigmine, as measured by PRs. However, in species with low levels of CaE, like the monkey, soman LD50s are low, and the full effect of pre-treatment with pyridostigmine can be detected (i.e., the PRs after pyridostigmine are high, and the clinical benefit is clear). Humans, according to the sponsor, have very low levels of CaE, and therefore, are expected to respond to pyridostigmine as robustly as do monkeys.

When Dr. Rosloff wrote his memo, the data on the relative amounts of CaE across species were obtained from several articles in the literature, and were obtained under varying experimental conditions; this made it difficult to reliably compare these reported values. Recently, however, the Army has performed new experiments which document, using standardized methodologies, that the relative amounts of CaE across species are as predicted. In these studies, (submitted on 1/13/03 and 1/14/03), a pair of plasma samples were obtained from the various species; one sample was treated with CBDP, a CaE inhibitor, and one sample was not treated. These samples were then compared for their level of binding of radiolabelled Soman. The following average concentration of binding sites blocked by CBDP compared to the non-CBDP samples (expressed in microMolar bound Soman, and interpreted as a measure of relative amounts of CaE) were demonstrated (there were 11-12 animals of each species tested, and 15 people tested):

Human	-0.022
Monkey	0.027
Guinea Pig	0.068
Rat	1.949

The standard deviations for the monkey, guinea pig, and human estimates were about 2-3 times the estimate, while the standard deviation for the rat was about half the estimate. These data establish that the amount of CaE in the monkey, guinea pig, and human are comparable and low, compared to the levels in the rat, which are clearly substantially greater than any of the other species tested.

Dr. Rosloff addresses the sponsor's points in his memo.

Regarding the point that there is a relatively constant increment in soman LD50 with pyridostigmine pre-treatment across species, data in monkeys, guinea pigs, and rats show the increment in soman LD50 due to pyridostigmine pre-treatment to be about 600, 136, and 175 mcg/kg, respectively. The sponsor rejects the value obtained for the monkey because it is a numerical outlier (with no further explanation).

As Table 4, page 4 of Dr. Rosloff's review notes, the increase in soman LD50 due to pyridostigmine in the rabbit ranges from 40-50 mcg/kg in two other studies. According to the sponsor, these low results are due to the fact that the dose of atropine was too low (below 16 mg/kg, a dose threshold not justified by the sponsor). The study by Koplovitz in 1992 (also described in Table 4), does not show any significant increase in soman LD50 in the rabbit.

Dr. Rosloff also notes additional studies that examined this point that were not cited by the sponsor. In particular, he notes a study in rats (described in his review of 7/24/96) in which the increment in the soman LD50 was only 10

mcg/kg, a study in which the dose of atropine was 16 mg/kg. He also cites 2 rabbit studies in which the soman LD50 increments were quite small, although the doses of atropine in these studies was also quite low (2 and 5 mg/kg).

He does also cite another study in the mouse, in which the increment in soman LD50 was 163 mcg/kg (which is very similar to the increments seen in the rat and guinea pig), but the control soman value in this study was based on an historical estimate. However, while it is usually treacherous to rely on historical controls, the control soman LD50 in the mouse is relatively stable.

Regarding the sponsor's theory that the relative levels of CaE are responsible for the inter-species differences seen in PRs, they provide data from studies that show that treatment with CBDP, an inhibitor of CaE, in these species does, in fact, result in a several-fold increase in the PRs after pre-treatment with pyridostigmine. This study was performed in rats (increase in the PR of about 4), rabbits, and guinea pigs (increase of the PR in both species of about 2). An additional study in mice showed a similar effect (increase in the PR of about 5), although, as Dr. Rosloff notes, that study did not include a group of mice not treated with CBDP; the lack of effect of pyridostigmine (on increasing the PR) in the absence of CBDP was based on data from earlier studies.

Of course, interpretation of these results cannot be definitive. In these studies, critically, the design did not include an atropine only arm; the studies only used pyridostigmine in conjunction with atropine. Therefore, the study was incapable, by design, of distinguishing the effects of the inhibitor on potentiating the effect of pyridostigmine alone. As Dr. Rosloff points out, however, some data do suggest that at the doses of CBDP used in these studies, atropine is not potentiated (a study utilizing much higher doses of CBDP did, however, show some potentiation of atropine) Studies of the effects of CBDP in species with presumably very low levels of CE (e.g., monkeys), in which no effect would be expected, have not been done. Further, the interpretation favored by the sponsor presupposes that CBDP has no important actions other than inhibition of CE. One can, again, imagine that CBDP could have multiple actions that could have contributed to the ultimate effect seen.

Dr. Rosloff does postulate a mechanism by which species with high levels of CaE might show little effect on the PRs, but, with pre-treatment with pyridostigmine, similar **increments** in the soman LD50s to those in species with low levels of CaE (see his review, page 6-7 for a detailed description of this scenario).

Briefly, he suggests (and performs sample calculations to support his conclusions) that if the reserve of CaE was large and saturable, pyridostigmine could have a small effect on the PRs, but still be effective, as measured by the increment in soman LD50, which would then be a valid measure of the effectiveness of pyridostigmine. As he points out, under this scenario, one would expect non-linear plasma levels of soman in species with high levels of CaE (low

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doses of soman would result in little to no detectable plasma levels, related to the "sink" effect of CaE; once this mechanism was saturated, plasma levels of soman would increase in a dose-dependent manner, resulting in an overall pattern of plasma levels that appeared non-linear). He cites one study comparing soman AUCs in rat, guinea pig, and marmoset, in which the authors conclude that plasma levels are linear in the pig and marmoset but not in the rat. My examination of the data suggests that, while Dr. Rosloff's conclusion that there are no strong deviations from linearity in any of the species is not unreasonable, (see Table 1, page 8 of his review), it is worth noting that only in the rat is there any evidence of non-linearity (I agree with him that individual animal data would have been useful).

The data in the rabbit is inconsistent, which is of some concern. Rabbits are presumed to have very low levels of CaE, but some studies suggest that they are not particularly responsive to pyridostigmine pre-treatment (that is, in these studies, they respond like species with high CaE levels), and the administration of the CaE inhibitor CBDP increased the soman LD50 by about the same multiple as it did in the guinea pig (about two-fold), which presumably has a somewhat greater amount of CaE than the rabbit (about 3 fold in one study). However, in the study by Maxwell, the PR in the rabbit was about 6, very similar to the PR for the guinea pig (about 5). This study, then, suggests that the rabbit and the guinea pig are relatively similar.

Relevance of Animal Endpoint to Human Endpoint

Clearly, the endpoint evaluated in the animal studies (survival) is directly relevant for the expected endpoint in humans, which is the same.

Dose Selection

The Army has selected a dose (30 mg every 8 hours) that results in a level of human RBC cholinesterase inhibition of 20-40%. As noted earlier, while we have good reason to believe that this dose does result in this degree of RBC cholinesterase inhibition, we also have good reason to believe that this is an inadequate marker of human (or animal) protection.

Dr. Booth, of the Office of Clinical Pharmacology and Biopharmaceutics, has performed a review in which he has determined that a human dose of 2.5-29 mg will provide plasma exposures (AUC) similar to those in monkeys that survived.

COMMENTS

In order to base the approval of this NDA on Subpart I, the elements of Subpart I must be met. This rule states that the mechanism of action of the toxic agent and the proposed treatment be reasonably well-understood, the efficacy must be

established in multiple species (unless a single species is clearly the appropriate model for humans), the endpoint in animals must be relevant to the human, and the data must allow the selection of an effective dose in humans.

Clearly, the endpoint in animals (survival) is the desired clinical endpoint. This is the only element of Subpart I that is met with certainty.

The mechanism of soman toxicity is, of course, not known with certainty, but can, I believe, be considered to be reasonably well-understood; namely, the irreversible inhibition of acetylcholinesterase resulting in excess, prolonged exposure to acetylcholine at various receptors, including the NMJ, with attendant respiratory failure (at least this mechanism is widely accepted as a major contributor to the cause of death). However, whether the mechanism of action of pyridostigmine in producing protection is "reasonably well-understood" is less clear.

I believe the answer to this question cannot be divorced from the larger question of the differential responsiveness of several other species to pyridostigmine. This is because, looked at in at least a certain way (i.e., by increase in PRs), pyridostigmine does not appear to be particularly effective in several species (rat, mouse, maybe rabbit), yet the presence, and action, of acetylcholinesterase and acetylcholine at the NMJ is ubiquitous across species. We can only hope to understand the mechanism of pyridostigmine's protection in those species sensitive to it if we understand its apparent lack of effect in insensitive species. In my view, this is the critical consideration in this application.

In addressing this question, it is important to consider that the primary, and generally considered to be the most important, pharmacologic action of pyridostigmine is as a reversible inhibitor of acetylcholinesterase. To be sure, it has other actions, some of which are known and undoubtedly others that are not. However, its well known action as a reversible cholinesterase inhibitor is clearly consistent with its proposed mechanism as a pre-treatment that protects against soman induced toxicity. If soman acts by irreversibly inhibiting cholinesterase, and pyridostigmine is, first and foremost, a reversible inhibitor of cholinesterase, it would appear to be reasonable to conclude that this action (in conjunction with atropine and 2-PAM) is primarily responsible for the animals' survival, as proposed by the sponsor. However, the conclusion that this is reasonable and consistent does not, in my view, **establish** this mechanism as reasonably well-understood. Further, and critically, as noted above, the mechanism cannot be reasonably well-understood in my view until we can explain the differential response (or lack thereof) in other species, specifically the rat, rabbit, and mouse.

In this regard, a number of points need to be made.

First, one can argue that the treatment is not ineffective in other species, as

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measured by PRs. For example, one rat study shows a slight effect, as measured by a PR of about 2 (although this is not a significant finding), and one rabbit study yielded a PR of about 6; however, other studies in these species and the mouse did not yield PRs substantially above 1.

However, the sponsor asserts that the absolute increase in the soman LD50 is a more appropriate metric by which to gauge the effectiveness of pyridostigmine. In this regard, studies show a relatively similar increase in the soman LD50 after pyridostigmine pre-treatment in the guinea pig, rat and mouse. The monkey demonstrates a much larger increase in the LD50, which the sponsor rejects because it is an outlier (it is greater than 3 standard deviations from the mean LD50 increase). My own view is that rejecting the value is inappropriate unless there is a compelling reason to believe that there was a flaw in the measurement procedure. However, I also believe that we need not be overly concerned that the value in the monkey is large; we would not necessarily expect the increase to be identical in all species. As Dr. Rosloff notes several times in his review, given the wide variations among species in multiple factors affecting a multitude of physiologic processes both identified and unidentified, it would be extraordinary, if pyridostigmine were effective in all, or most, species, for its effect to be quantitatively similar in these species, regardless of the metric used. I agree that the increase in soman LD50 suggests that the treatments are effective in the rat; although the degree of effectiveness is presumably not necessarily of the magnitude that, were it to occur in humans, would be particularly effective.

As noted earlier, the sponsor posits that high levels of carboxylesterase, an enzyme that rapidly clears soman, is responsible for the "apparent" lack of effect in the rat, mouse, and rabbit, as measured by PR. Recent data generated by the Army confirms that monkeys and guinea pigs have very low levels of CaE, as do humans, and the Army concludes that these findings permit the conclusion that that humans will respond to pyridostigmine pre-treatment as do monkeys; namely that humans will have a large increase in PR, and hence survival.

Dr. Rosloff has described a scenario in which similar increments in soman LD50 across species, in the face of discrepant PRs, would be possible (in other words, he provides a mechanism to explain the results seen). His scenario depends upon, as proposed by the sponsor, the presence of (relatively) high levels of carboxylesterase in those species with low PRs. Although his proposal is theoretical, and his calculations are a manufactured example, I find the explanation compelling, primarily because there is, in the relevant species, a presumably fixed pool of CaE, and CaE's capacity to inactivate soman is well accepted. Given these factors, it is difficult to imagine that the system functions in any way other than that posited by Dr. Rosloff.

Several lines of reasoning support the sponsor's points.

First, the sponsor's assertion that rats have high levels of CaE, and that monkeys, guinea pigs, and humans have low levels, has recently been confirmed (when Dr. Rosloff wrote his review, the Army had not submitted reliable data from a single study comparing levels of CaE in the various species). It should be noted that rabbits have somewhat lower levels than guinea pig (the findings in the rabbit are somewhat inconsistent; see below).

Next, the sponsor presents the results of studies that purport to demonstrate that when CaE is inhibited in the rat, rabbit, guinea pig, and mouse, the PRs are increased; that is, these species begin to respond to pyridostigmine pre-treatment like the monkey, a species with very low levels of CaE. These data are critical to the sponsor's argument about the mechanism of action of pyridostigmine and deserve close inspection.

Indeed, I believe that the issue of the apparent differential response to pyridostigmine pre-treatment among species, and the Army's explanations of these differences, is the critical issue in the application, because, in the absence of a compelling explanation for this apparent difference, this finding raises doubts about pyridostigmine's proposed mechanism of action, and therefore, critically, whether or not the animal data (in particular, the monkey data) can reasonably be expected to predict the human response. In this context, the CBDP data are critical, but there are a number of concerns about this data.

Specifically, in order for us to consider this data highly supportive of the sponsor's claims, it is crucial that we be able to conclude that CBDP acts by inhibiting CaE, and that it has no other important actions, especially that it is not acting by enhancing pyridostigmine's effects. If CBDP were to be acting (that is, increasing the PRs in those species with high levels of CaE) to any important degree other than as a CaE inhibitor, this would raise serious questions about the sponsor's proposed explanation for the low PRs in non-CBDP treated species with low CaE levels.

With respect to the inhibition studies, however, I first have several comments about Dr. Rosloff's conclusions.

Dr. Rosloff notes that the sponsor has submitted a paper which demonstrates that the PR is increased in mice who have been treated with the inhibitor, but that there were no groups in the study not treated with the inhibitor; in this case, the sponsor's conclusion is supported by reference to a historical control. My view is that this is not a fatal flaw in the study; the lack of response in the mouse without treatment with the inhibitor is, in my view, fairly well established.

Beyond this, Dr. Rosloff notes that these studies could not, by design, determine if the inhibitor potentiated pyridostigmine, atropine, or both. I am convinced that, although Dr. Rosloff is obviously correct, other data sufficiently establish that atropine is not importantly potentiated by the inhibitor.

With regard to these studies, then, it is interesting to note that the degree to which treatment with the inhibitor increases the PR in the rat (about 4 fold) is not much different than the increase in the rabbit and guinea pig (about 2 fold in each). The relative similarities in these increases, despite a greater than a degree of magnitude difference in the CaE levels, suggests that there may be actions of CBDP other than pure inhibition of CaE.

(To digress somewhat at this point, although the animal rule requires a reasonably good understanding of the mechanism of the effect of the treatment in animals, one could interpret the data using a more straightforward, empirical approach.

Specifically, one could simply observe that the several species in which pyridostigmine seems to be minimally or not effective have high levels of CaE, and, that when one inhibits the CaE in these species, pyridostigmine appears to confer a benefit [the degree of which may vary among species]. While this approach does not presuppose a detailed understanding of the mechanisms involved [something Subpart I does seem to require], it does provide a reasonable rationale for predicting that species with low CaE levels [including, and especially, humans] will respond to pyridostigmine pre-treatment similarly [again, the degree of response cannot be predicted with great certainty]. However, as Dr. Rosloff points out, reaching this conclusion from this empirical approach still would require that actions of CBDP other than inhibition of CaE would need to be ruled out. I see no escape from this conclusion.

It is worth noting that I find this empirical approach particularly attractive, because the interpretation is simple and straightforward; if species have similar levels of active CaE, they will respond similarly to pyridostigmine pre-treatment. While one could argue that such an approach does not fulfill the requirement in Subpart I to have a reasonably well-understood mechanism of toxicity/drug action, it seems to me that the very fact of the response [as measured by increased PR] to CBDP in the "insensitive" species provides a more than reasonable basis for concluding that CaE levels are involved in the response, and that species with similar naturally occurring levels of CaE should respond to pyridostigmine pre-treatment similarly, although I would not necessarily expect that they would respond [quantitatively] identically. It is, of course, possible that this interpretation is incorrect, but I believe that, if we can reliably conclude that CBDP has no other important activity other than CaE inhibition, I would be comfortable reaching this conclusion.)

While, as Dr. Rosloff notes, these data are not necessarily definitive, I believe that the Army has established that the critical action of CBDP in this regard is reasonably well-understood.

First, one study found no effect of CBDP on acetylcholinesterase, providing some direct evidence of a lack of effect on another enzyme that clearly binds CaE.

One apparent troubling aspect of the data that is relevant to this point relates to the degree of increase in the PR seen across species in the Maxwell study. Maxwell found, as described above, treatment with CBDP increased the PR by about 4 in the rat, and by about 2 in the rabbit and guinea pig. Given the relative amounts of CaE between these species (especially the pig and rat), we might have expected to see a much greater increase in the PR of the rat relative to the guinea pig. This is somewhat troubling because, as noted earlier, if there is no material difference in the increase in the PR between the rat and guinea pig, this would cast doubt on the CaE-based explanation of differential responsiveness to pyridostigmine across species, and hence would cast similar doubts on the prediction that humans will respond similarly to monkeys. To further establish that CBDP is increasing the PR via the mechanism of CaE inhibition, two approaches might be fruitful.

First, as Dr. Rosloff suggests, a showing that CBDP treatment does not materially increase the PR in the monkey, the species in which the greatest efficacy has been established, and which is a low-CaE species, would strongly support the view that CBDP is working through CaE inhibition. This study, of course, has not been done. However, given the recent data on the relative amounts of CaE in monkey and guinea pig cited above, one could argue that a CBDP study in guinea pigs could provide the same information (as noted, this study has been done by Maxwell, and it demonstrated only a two-fold increase in the PR).

However, I believe an alternate approach would be to replicate the finding in the rat. As I noted above, the relatively small increase in the PR in the rat compared to that in the guinea pig raises the question about whether this difference is real or not (if it is not, the support for the Army's theory is considerably diminished). However, if a difference of a similar magnitude were detected on replication, this would strongly suggest that the finding was "real". At that point, I would be less concerned about the "small" difference on the effect on the PR in the rat compared-to the guinea pig. In reality, although the amount of CaE in the rat is many multiples of that in the guinea pig, I have no way of knowing how the difference in the CaE levels should relate, quantitatively, to any "expected" increase in the PRs between the species. (It should be noted that the 4-fold increase in the PR in the rat resulted in a PR of about 8, while the 2 fold increase in the PR of the guinea pig resulted in a PR of about 10; the similarity of these ultimate PRs in the two species might suggest that the maximum effect of pyridostigmine had been obtained.)

Having stated my view that replication would suffice to establish that CBDP is functioning primarily via CaE-inhibition, then, I would suggest that the Army has provided a degree of replication already. Specifically, I suggest that the data in

the mouse noted earlier provide a considerable degree of replication on this point. In particular, the levels of CaE in these two species are quite comparable (one study by Maxwell documented levels of 4.2micM in the rat, and 6.1micM in the mouse), and the increase in the PR in the CBDP-treated mouse was about 5 (albeit compared to a historical control which, I noted earlier, I consider fairly well established). This similarity in the increase in the PR of the mouse and rat, two species with quite similar CaE levels, provides, in my view, a considerable degree of "replication" and therefore considerable support for the sponsor's contention that CBDP is primarily working via CaE inhibition (indeed, the quantitative results in the mouse compared to the rat are also consistent with prediction; that is, the mouse, with the slightly greater CaE levels, would be expected to show an increase in the PR somewhat greater than that in the rat, a prediction borne out by the data [although, given the vagaries of the data, I would not make too much of this finding]).

Finally, on this point, additional data further suggest that CBDP is not working through enhancement of pyridostigmine.

In particular, a comparison of the Soman LD50's in 4 treatment groups (no pyridostigmine pre-treatment, pyridostigmine pre-treatment, and each of these groups with CBDP) is instructive in this regard.

Maxwell, in the same paper in which he examined the change in PR in CBDP-treated animals, showed that the difference in Soman LD50 between the no pyridostigmine pre-treatment (control group) and the pyridostigmine pre-treatment was essentially the same as the difference in Soman LD50 between the CBDP-treated control group and the CBDP-treated pyridostigmine pre-treatment group for three species (rat, guinea pig, and rabbit). The data are shown below:

Species	Delta Soman LD50 Without CBDP	Delta Soman LD50 With CBDP
Rat	136 mcg/kg	117 mcg/kg
Guinea Pig	118 mcg/kg	111 mcg/kg
Rabbit	101 mcg/kg	102 mcg/kg

The constant delta implies an action of CBDP "alone", that is, through a mechanism other than enhancement of the effect of pyridostigmine. For this reason, these data support the proposed mechanism of action of CBDP in this study (i.e., CaE-inhibition).

Equally critically, our understanding of the species differences relies upon our information about the relative amounts of CaE among species. As I have noted previously, the sponsor's recent submissions have convinced me that the relative

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amounts of CaE across species described in the literature have been adequately confirmed.

Dr. Rosloff has recommended that the application be judged Not Approvable until additional data are presented. Specifically, he recommends that the CaE levels in the various species be better documented, and that the study in which CBDP was administered should be replicated and extended to verify the result in the mouse, rule out that CBDP potentiated the effects of atropine, administer CBDP to monkeys to document the lack of effect that is predicted, and measure soman levels. He also recommends that the sponsor address the anomalous results in the rabbit. He makes these recommendations because he suggests that these studies are critical to support the sponsor's explanation of the species differences.

I, of course, agree that the question of differential response across species is the critical question that needs to be addressed. However, as I have noted above, I am less concerned about these aspects of the data than is Dr. Rosloff. Specifically, I do not believe that it is essential, at this time, for the sponsor to verify the result in the mouse, rule out a potentiating effect of CBDP on atropine, or measure soman levels, for the reasons I have already given. As I have described earlier, I believe that the existing data, while not perfect, are sufficient to convince me that these specific issues have been adequately addressed.

With regard to the rabbit data, I have the following comments.

I noted earlier that the data in the rabbit are somewhat inconsistent.

Specifically, some studies show that the PRs in the rabbit are very low (less than 2), though the levels of CaE are about one half-one third that in the guinea pig, which has relatively high PRs (4-6). However, a study by Maxwell did demonstrate a PR in the rabbit of about 6; in this study, the PR in the guinea pig also was about 5, consistent with other estimates of the PR in the guinea pig. Further, Subpart I does not require absolute consistency in response across all species (although such consistency would be ideal), and, if we found the rest of the data in other species to be consistent, the lack of consistency of the rabbit findings with the other species might be dismissible. However, given the finding of a PR of 6 in the one study (a study, again, which identified a PR for the guinea pig consistent with previous estimates of the PR in the guinea pig), and the relative similarity of CaE levels in guinea pigs and rabbits, I am convinced that there is at least some evidence of a similarity between the guinea pig and rabbit (again, even if the evidence in the rabbit was inconsistent with the evidence in all other species, this would not, as described above, preclude the use of Subpart I), and that, therefore, the findings in the rabbit do not, in my view, present a significant bar to approval of the application under Subpart I. In this regard, it is worth noting that in the 2 studies that Dr. Rosloff describes that are essentially

negative in the rabbit (see his review, page 5), the doses of atropine were relatively low compared to doses in other studies.

Regarding the appropriate dose to be given to humans, I noted earlier that Dr. Booth has calculated a human dose expected to result in plasma exposures (AUC) associated with increased survival in the monkey. The upper limit of the dose (29 mg) is essentially identical to the sponsor's proposed 30 mg every 8 hour regimen.

I am not entirely sure that AUC is the appropriate kinetic measure with which to compare the species (and hence choose a human dose). The pharmacokinetic/pharmacodynamic relationship that confers benefit in the monkey (or any other species) is extraordinarily complex, and, I would argue, poorly understood. For instance, one view (the one under which we have largely been proceeding) is that pyridostigmine confers benefit only when its levels are decreasing because it is at these times that functional cholinesterase is being released (it should be noted that this is not the only view about the PK/PD relationship).

While comparing the kinetics in the monkey and human (as a basis for the choice of human dose) is one potential approach, my view is that the dose that will provide the maximum amount of cholinesterase protection that can be safely achieved seems to be a fair basis for the choice of the dose in humans (simply, all things being equal, the more enzyme that is protected, the more that can, theoretically, be released and be functional after the soman is cleared). Given this, I believe that the sponsor's proposed dose is acceptable; it is well tolerated, and higher doses may be considerably less well-tolerated.

Finally, regarding safety, the relevant data have been previously reviewed. I am aware of no data that otherwise healthy young adults should not be able to, in general, tolerate this dosing regimen. Long experience with much greater doses in patients with myasthenia gravis supports this conclusion, and the previous experience in Operation Desert Storm, at the doses proposed here, does as well. For this reason, I believe the proposed dosing regimen is acceptable.

Other issues

In the January 3, 2003 NDA re-submission, the sponsor addressed a number of issues that were discussed at a 12/19/02 meeting between DoD and the Agency. These issues have been addressed in the reviews of Dr. Rzeszotarski, Dr. Prohaska, and Dr. Kavanagh. I will briefly review these issues.

Stockpiling

The Army has considerable stocks of packaged pyridostigmine in place for use under the IND. This product has been manufactured by Roche, ―― and ICN, whereas the manufacturer of the product covered in the NDA is ICN. The current product consists of a blister pack of 21 pyridostigmine tablets in a cardboard sleeve; in addition to the drug, the sleeve contains a patient information sheet. There is some information printed on the outside of the cardboard sleeve, including that the use of the product is investigational.

The Army asserts that it will be practically and logistically impossible to immediately replace currently stockpiled product with the NDA approved product and packaging. They have submitted a plan for the phasing in of new product and new packaging.

In brief, they propose to immediately (as soon as possible) add a small laminated card into the sleeve that will explain that the product is now approved by the FDA, the approval is based on animal data, and directs the soldier to read the enclosed safety sheet. Further, they propose to re-package all of the product within 2 years; this will include a new cardboard sleeve with new wording, and the new patient safety sheet. Over the next five years, the entire currently stockpiled product will be replaced with new product approved under the NDA, with the oldest stockpiled product to be replaced first. Therefore, within 5 years of the approval of the NDA, all product will be NDA product, with approved labeling and carton (sleeve) label.

This proposal is acceptable.

Expiration dating

The sponsor has proposed a 10 year expiration. This is, obviously, an unusually long expiry, but a long expiry appears warranted for this product. Dr. Rzeszotarski has concluded that such an expiry is acceptable, based on extended stability data for the Roche ―― product, and the similarity between these products and the ICN product (the Roche ―― products have been permitted extended expiration dates under the Agency's Shelf-Life Extension Program). The 10 year expiry will need to be supported by yearly stability testing after approval, and Dr. Rzeszotarski also recommends that the sponsor submit (within one month of approval) a stability protocol for the initiation of this stability program.

In addition, Dr. Rzeszotarski recommends that the bulk and secondary containers include the following statement:

The contents are not to be used if the package is removed from refrigeration for more than a total of six months.

Further, he recommends the following statement be placed on the blister pack:

Discard after three months of use.

These proposals are acceptable.

Post-marketing studies

Subpart I requires that studies designed to establish the effectiveness of the product in humans be performed, if feasible, and that plans for these studies are to be submitted in the application.

The Army has submitted four protocols, and they have been reviewed by Dr. Prohaska.

Briefly, the first study will retrospectively compare adverse effects of soldiers who did, and did not, take pyridostigmine, at selected units.

The second study will be a retrospective survey of health care providers who are involved in the treatment of nerve agent casualties. Presumably (although this is not clear), the intention is to compare, in soldiers exposed to nerve agents, the outcomes in soldiers who did, and who did not, take pyridostigmine.

The third study will compare the survival data using various statistical techniques incorporating a collection of relevant data (see Dr. Prohaska's review of 1/9/03, page 2).

Finally, the fourth study will again evaluate adverse events in soldiers who did, and did not, take pyridostigmine; the difference between this proposal and the first described is not clear.

As Dr. Prohaska notes, these studies are not described in detail, although only the second and third appear to be responsive to the requirements of Subpart I. I believe that the sponsor should be told that detailed protocols for such studies should be submitted as a Phase 4 commitment.

Patient Information

Subpart I requires that patients be given a patient information sheet. The sponsor has submitted a version, which we have modified, and is included in the package. They have also prepared an "interim" information sheet, which is the laminated card described above.

Chemistry

We had asked for a number of items to be submitted in the CMC portion of the NDA. All of the requested information has been submitted, and has been reviewed and found acceptable by Dr. Rzeszotarski. In addition to the comments noted above about statements for packaging, Dr. Rzeszotarski also recommends that the sponsor commit to deliver the methods validation package one month from the date of approval, and to initiate a long-term stability program, to include yearly stability testing out to 10 years (see above).

These recommendations are acceptable.

Biopharmaceutics

The Office of Clinical Pharmacology and Biopharmaceutics requested information pertaining to the batches used in a pivotal bioequivalence study comparing the Roche product with the ICN product. Dissolution data were requested as well.

The sponsor has submitted the requested information, and Dr. Kavanagh has found it to be acceptable. In particular, an interim dissolution specification has been set, and the sponsor has made a commitment to provide additional dissolution data to support a final, definitive dissolution specification.

These proposals are acceptable.

Package Insert

We have included our version of the package insert in the package. The Army has proposed a plan for insuring that this insert is distributed to the relevant Army personnel via e-mail at the time of approval. This plan is acceptable.

Additional studies

As we have discussed with the Army, the on-going and planned studies designed to validate in animals the newly proposed surrogate discussed earlier are expected to be completed. In the approval letter, we will include this development program as a Phase 4 commitment.

CONCLUSIONS

The Army has presented a comprehensive package of data that purports to establish that the criteria of Subpart I have been met, and they therefore conclude that the NDA may be approved at this time.

There is no doubt, of course, that the data presented are not completely consistent (for example, some studies show an important increment in soman LD50 in the presence of pyridostigmine pre-treatment in the rat, and others do not; some of the negative studies are explained by the sponsor as the result of low atropine levels [with little explanation about how they know that these levels are too low], while some negative studies employed levels the sponsor accepts as appropriate). Further, the meaning of a "large" absolute increase in the soman LD50 with pyridostigmine pre-treatment (compared to the LD50 without pre-treatment), as opposed to a large PR, vis-à-vis survival, especially in species with high baseline CaE levels, is also not completely clear (for example, it is possible that this degree of an absolute increase in high CaE species confers a benefit equivalent to a large PR in low CaE species; were this true, it would provide additional support to the sponsor's position that pre-treatment is effective in these species, although, again, according to the theory, such an increase in the increment in Soman LD50 in low-CaE species, like humans, would not be expected to confer an important benefit).

Nonetheless, given these inconsistencies, I do believe that, taken as a whole, the data do support the sponsor's conclusion that the requirements of Subpart I have been fulfilled.

I believe that the sponsor has presented data that supports the conclusion that the mechanism of soman toxicity is reasonably well-understood, and that the mechanism of pyridostigmine protection is also reasonably well-understood. This latter, as I have tried to establish, is, in my view, inextricably related to the sponsor's explanation for the disparity in response to pyridostigmine pre-treatment among the various species, as measured by the PR. As noted, this explanation is critical to our ability to extrapolate the monkey findings to the human.

In particular, I believe that the data support the view that the differences among species in this regard are adequately explained by the relative amounts of CaE in the various species. I believe that the studies in which CaE is inhibited by CBDP provide strong evidence that the relative amounts of CaE explain the species differences in PRs. Critical to this interpretation are the data that speak to the question of whether or not CBDP has important effects other than inhibition of CaE in these studies. In response to this question, taken as a whole, the evidence (the absence of a demonstrable effect of CBDP on acetylcholinesterase as well as, critically, the absence of enhancement of the effect of pyridostigmine, in conjunction with the data in the mouse, which, in my view, establishes

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replication of the rat finding, and serves to validate the degree of the relative increases in PRs seen between rat and guinea pig), adequately supports the conclusion that the effect of CBDP in these studies was due largely (if not exclusively), to inhibition of CaE.

In addition, the sponsor has established, to my satisfaction, the relative levels of CaE among species, including humans. This, of course, is also a critical aspect of the sponsor's proposed mechanism of action arguments, and of their prediction about human responsiveness.

It is important to point out that no element of Subpart I (save for the requirement that the animal endpoint be relevant for the desired human endpoint) has been definitively demonstrated to have been met. Additional studies could be performed to further address all of the remaining elements. For example, we could require, as Dr. Rosloff suggests, a study designed to demonstrate the effect on the PR of CBDP treatment in the monkey. We would expect no (or negligible) effect, a finding which would further confirm that CBDP had no effect other than CaE inhibition. We could require the exploration of the effects of pyridostigmine pre-treatment in other animal species, in an attempt to determine if the results in these species are consistent with the results seen here. Indeed, all of the studies recommended by Dr. Rosloff (including the independent replication of several of the studies presented here, better characterization of the effects in the mouse and rabbit, and studies to better understand the effects of CBDP on atropine) are reasonable, and would help to confirm the results presented. One could imagine any number of studies that could be designed that could provide additional support for the sponsor's proposed mechanism.

Ultimately, of course, none of the sponsor's explanations can be definitively established. The drafters of Subpart I understood this fact; the standard they chose to adopt was that the relevant mechanisms and biological and pharmacological events be only reasonably well-understood. This standard, of course, is open to personal interpretation; the quality and quantity of data found to have met this standard will, of necessity, vary from person to person. In this memo, I have tried to explain why, despite the deficiencies, I believe that the data, taken as a whole, support the view that the mechanism of soman toxicity and the mechanism of pyridostigmine's protective effect, are reasonably well-understood. As a result, I believe it is reasonable to conclude that humans will respond similarly to monkeys, and that the treatment, therefore, is likely to be effective in humans.

It is worth noting, finally, that, even under the best of circumstances, there will be much that the animal studies cannot tell us about how pyridostigmine pre-treatment will work in humans under real battlefield conditions, even given that I believe that the animal data support the conclusion that it is likely to be effective in humans. For example, as Dr. Rosloff explains, the studies in animals were done so that the soman exposure (which, incidentally, was not administered via

the route that people will be exposed to in the battlefield) was given at an ideal time in relation to pyridostigmine pre-treatment, and there is no information about the effect of pre-treatment in the face of a persistent exposure to soman. However, I do not find this particularly damaging, although it would be useful to have this information. The animal studies may be seen as providing a proof of principle that the treatment is effective under certain circumstances; this is no different from the typical product approved on the basis of clinical studies. I see no practical way for the Army to demonstrate that the treatment will be effective under all possible battlefield conditions.

Finally, we have negotiated final labeling with the Army, and we have obtained their commitment to provide the additional data discussed above.

For the reasons given above, then, I recommend that the Agency issue the attached Approval letter with the appended labeling.

APPEARS THIS WAY
ON ORIGINAL

MEMORANDUM

Date: January 10, 2003

To: Dr. Russell Katz
                 Director
                 Division of Neuropharmacologic Drug Products
                 HFD-120

From: Lisa Stockbridge, Ph.D.
                 Regulatory Reviewer
                 Division of Drug Marketing, Advertising, and Communications
                 HFD-42

Re: NDA 20-414
                 Pyridostigmine Bromide Tablets

Material Reviewed: January 9, 2003 proposal of Patient Information Fact Sheet (PPI) for military
                              use

Recommendations:

The bulleted format is recommended because it is easier to read quickly in emergency or stressful situations.

Use the term "effectiveness" (with definition) rather than "efficacy" for better patient understanding.

I suggest the following revisions to the format and content of the PPI:

_____1_____ page(s) of
revised draft labeling
has been redacted
from this portion of
the review.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Lisa Stockbridge
1/10/03 09:58:38 AM
CSO

MEMORANDUM

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

DATE: January 10 , 2003

TO: Robin Nighswander, Chief, Project Management Staff
Division of Neuropharmacological Drug Products
HFD-120

FROM: Jeanine Best, M.S.N., R.N., P.N.P.
Patient Product Information Specialist
Division of Surveillance, Research, and Communication Support
HFD-410

THROUGH: Anne Trontell, M.D., M.P.H., Director
Division of Surveillance, Research, and Communication Support
HFD-410

SUBJECT: ODS/DSRCS Review of the Patient Labeling for Pyridostigmine
Bromide, NDA 20-414

The Patient Labeling which follows represents the revised risk communication materials for
Pyridostigmine Brominde, NDA 20-414. It has been reviewed by our office and by DDMAC.
We have simplified the wording and made it consistent with the PI.

APPEARS THIS WAY
ON ORIGINAL

Memorandum   Department of Health and Human Services
Public Health Service
Food and Drug Administration
Center for Drug Evaluation and Research

DATE: September 18, 1998

FROM: Director,
Division of Neuropharmacological   Drug Products
HFD-120

SUBJECT: NA Action on NDA 20-414

TO: File NDA 20-414
&
Director, ODE-1
HFD-101

My June 24, 1998 memorandum to the administrative file explained why I found there to be a lack of substantial evidence to support the DOA's claim that pyridostigmine is effective for use as a component of a multi-drug regimen intended to enhance survival and lessen morbidity among individuals exposed to cholinesterase nerve agents.   This memorandum serves, therefore, not to address evidence bearing on the proposed regulatory action, but to challenge an assertion offered in an 8/20/98 memorandum issued by the ODE 1 Office Director concerning the reasons I offered to support a not approval action.

The Office Director's memorandum of 8/20/98 asserts that I wrote on page 2 of my 6/24/98 that animal data could "never" be used as a basis for reaching a conclusion under subpart H of part 312.   That assertion is incorrect.   My memorandum does not speak to what is allowed by that regulation, but to why, from an epistemological and scientific perspective, I believed it would be imprudent to extrapolate from an effect observed in an animal model to a conclusion about the effectiveness of the intervention in humans.

/S/

Paul Leber, M.D.
September 2, 1998

cc:
NDA 20-414
HFD-101
     Temple
HFD-120
     Katz
     Tresley
     Rosloff
     Fitzgerald
     Nighswander

M E M O R .:` N ᴊ U M   DEPARTMENT OF HEALTH AND HUMAN SERVICES
                                      PUBLIC HEALTH SERVICE
                                    FOOD AND DRUG ADMINISTRATION
                            CENTER FOR DRUG EVALUATION AND RESEARCH

DATE: August 20, 1998

FROM: Director, Office of Drug Evaluation I

SUBJECT: NDA 20-414, pyridostygmine bromide (PB)

TO: File NDA 20-414

HFD-120 has forwarded a NA recommendation for NDA 20-414 for PB to be used as
prophylaxis against nerve agents (Soman, in particular). I do not agree with what
I read as Dr. Leber's essentially absolute conclusion that animal data linking
blood cholinesterase inhibition by PB to protection against nerve agents together
with human evidence of blood cholinesterase inhibition, could never be taken as
evidence of effectiveness under subpart H of part 312 (page 2, memo of 6/24/98).
The preamble to the final subpart H rule in citing animal data as part of the basis
for considering that a cholesterol-lowering/effect could be a basis for drug
approval and makes it clear that animal data could be a basis for concluding that a
surrogate predicts clinical benefit. As I wrote that part of the preamble, I agree
with it. Whether animal data are, in a particular case, "reasonably" likely to
predict clinical benefit, is of course, another matter.

I should also note that there is nothing unusual (Leber memo, page 3) in treating a
"rebuttal" of a nonapprovable letter as a resubmission (even without new data);
In any event, despite accepting the possibility that animal data could support a
surrogate, I concur with the Division's view that human blood cholinesterase
inhibition has not been shown to be reasonable surrogate for the hoped for survival
benefit. The reasons for this conclusion are fully explained in the letter.

                              /S/

                    Robert Temple, M.D.