Notes of discussions with Lewis Smith to brief him on the latest Parkinson's disease findings on 3rd December 2004.

Present: Lewis Smith, Nick Sturgess, Louise Marks and Mike Clapp

Nick and Louise presented the latest findings – presentation on file 'I' drive Paraquat/PD

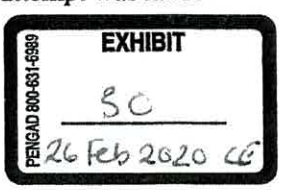
1. Is the loss of neurones indicating generalised neuronal toxicity in the brain rather than a specific effect in the Substantia Nigra? This is not entirely clear, however data from other brain areas (e.g. hippocampus) suggest that it is specific, but it may be difficult to detect changes in dopaminergic neurone number in other brain regions where the density of dopamine containing neurones is lower than in the SNpc, thus making a loss of neurones more difficult to detect.

2. Is the C57Bl6 more sensitive than other strains and species? It is the most commonly used strain although some researchers have used other non-pigmented mouse strains including Swiss-Webster, CF1 & BALB/c mice, and C57Bl6 do appear more sensitive to MPTP. The rat appears to be less sensitive, with no clear difference between black Norwegian and albino rats reported in the literature although it is unlikely that this would have been examined in detail owing to the poor neurotoxicity observed with MPTP dosed to the rat.

3. If part of the reason that the C57Bl6 mouse is more sensitive to MPTP is the role of melanin then is there any evidence for melanin to make a difference in the human population? The incidence of PD in black individuals or of African origin was addressed in the MRC IEH Review on *Pesticides & Parkinson's Disease: -*

"Prevalence of PD is generally considered to be highest in nations with predominantly white populations. However, this does not necessarily equate to a higher PD risk amongst whites. A recent incidence study of PD in northern California, USA found that age- and sex-adjusted PD incidence was highest amongst Hispanics, followed by non-Hispanic whites, Asians and blacks. However the differences between individual groups were either not statistically significant (p=0.05) or only of borderline significance. Other incidence studies have also failed to find a difference between ethnic groups. For example it has been found that men of Japanese or Okinawan ancestry residing in Hawaii, USA experienced PD risks of the same pattern and magnitude as Caucasian men in Europe and the USA, and higher than Asian men living in Asian nations. Additionally, PD prevalence amongst blacks in Mississippi, USA (many of whom descended from populations in West Africa) has been found to be similar to that of the white population in Mississippi (341 and 347 per 100,000, respectively), and substantially higher than blacks in Nigeria, West Africa (67 per 100,000). This suggests that ethnicity may not be a risk factor in PD, but rather that environmental and / or lifestyle factors may have a greater influence on the risk of developing PD".
Also a 2004 review of the prevalence of PD in populations of African ancestry concluded that any apparent differences previously reported were unproven, and well designed studies were required to determine whether any previously reported differences were indeed real (McInerney-Leo *et al* 2004, J Natl. Med. Assoc. **96** (7): 974-979.)

4. The total dose of MPTP received was greater than that of the paraquat dose (200mg vs. 30mg) and this may raise some questions relating to the PQ dose required to produce an effect is lower than that of MPTP. Although this is factually correct, no attempt was made



to compare potency of the two chemicals. In this instance we were deliberately using as high a dose of MPTP as possible to produce the maximum effect. It is also worth bearing in mind that clinical signs of neurotoxicity are observed with MPTP but not with PQ, so PQ is not more toxic than MPTP.

5. LLS recommended that NS & LM consult widely on the methodology to test if there could be any other explanations for observing a lower number of neurons. It maybe worth consulting the Karolinska Institute. He also suggested that 1 micron sections could give a benefit, although it is difficult to see why using very thin sections would help, since 1 um sections will contain parts of the same neuron over several serial sections leading to the counting of the same neuron more than once. The whole point of thick (40 um) sections is to avoid this issue and all the neurons within a section sample area are only counted once – whole basis upon which stereology is based.

6. It was agreed that the sensitivity of the computer model for estimating loss of neurons needed to be checked by varying the input parameters. What if, for example, the initial thickness of the original section was not cut at 40 um thick in all cases? **Action LM to talk to Micro Bright Field Inc about how these parameters would affect total counts.**

7. LLS mentioned that from memory paraquat causes diuresis in the mouse, which could have resulted tissue becoming dehydrated and this may have altered cell count. This will be considered.

8. . In studies with MPTP and PQ, the neurons that have survived seem to be Calbindin positive, implicating a neuroprotective role for this Ca^{2+} binding protein.

9. It was suggested by LLS that the effects of a diuretic to investigate the effect of dehydration should be determined in a study in order to see if this results in an apparent neuronal cell loss in the same way as with PQ. He suggested that with the appropriate use of [^{3}H]-H_2O and [^{14}C]-inulin, one could determine whether there has been intra or intercellular compartment dehydration as a result of PQ exposure, which in turn may have influenced neuronal counts.

10. LLS also suggested that it might be useful to look at the effect of time post final PQ dose on the extent of the neuronal cell loss – does it recover over a period of weeks. If not, this will suggest that the 21% reduction is permanent and not temporary. Durations of 7, 14, 28 and 90 days post dose were briefly discussed, but no firm recommendations made.

Conclusion there is no block on following up on methodological issues and these should be challenged aggressively. HA have a responsibility to create the scientific understanding and there will be no intention to slow down this understanding, although business risk will need to be considered in the decision making process. The next step is to present the overall Strategy to the Health Assessment Technical Committee on 16th Dec, after which LLS requested that future direction of the research should be presented to the regulatory Sciences Committee and that he would create a teleconference for this to occur around the turn of the year.

MJLC/NS
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