

How does the herbicide paraquat enter the brain?

Paraquat (1,1'-dimethyl- 4,4'-bipyridium) is an organic di-cation at physiological pH and will not readily cross cell membranes. However, paraquat has been shown to concentrate in lung cells, where it enters via a polyamine transport system and in kidney cells where it undergoes active transport via an organic cation transport system. These transport systems are not species specific have been observed in experimental animals such as rats, mice, dogs and monkeys as well as in humans.

Recent studies have demonstrated paraquat entry into the brain of a particular strain of mouse C57Bl6 where it causes selective injury to dopaminergic neurones in the substantia nigra as judged by loss of neurotransmitter, reduced neuronal cell counts and behavioural changes (Brooks et al., 1999; Thiruchelvam et al., 2000; McCormack et al., 2002). Surprisingly, it also has been shown that neutral amino acids such as L-valine when administered to mice at the same time as paraquat will reduce paraquat entry into the substantia nigra and prevent the neuronal cell injury (Shimizu et al., 2001; McCormack et al., 2003). While the amino acids D-valine or L-lysine had no effect. This data suggests that paraquat may enter the brain via a neutral amino acid transport system, which is very surprising and currently cannot be rationalised, although in time an explanation will no doubt be found.

What is the evidence to support the notion that paraquat enters cells via a organic cation transport (OCT) system? Three OCT's have been isolated, cloned and expressed in cells and characterised. OCT1 was first cloned by Grundemann et al., (1994) and subsequently its substrate specificity and electrogenic properties determined in rat and human kidney. OCT1 is responsible for the uptake of a wide variety of organic cations (OC) into epithelial cells such as renal tubule cells and hepatocytes. OCT-1 transports OC such as MPP⁺, TEA, quinine, choline and polyamines such as spermine and spermidine, while paraquat is poorly transported. OCT1 is also expressed in murine kidney (Mori et al., 1997; Mooslehner & Allen (1999). Okuda et al., (1996) cloned, expressed and characterised a novel rat kidney OC transporter, OCT-2. OCT-2 was shown to stimulate the uptake of TEA, and this was markedly inhibited by cimetidine, procainamide and quinidine. Human homologues of OCT1 and OCT2 have also been cloned and functionally characterised (Gorboulev et al., 1997; Zhang et al., 1997), and OCT2 has been reported in murine kidney (Eraly et al., 2003). Okuda et al (1998) then isolated a cDNA from rat placenta, which when expressed heterologously, mediates the transport of a wide spectrum of OC. This was designated OCT- 3, and found to be abundantly expressed in the placenta and moderately expressed in the intestine, heart and brain. Three other OCT members OCTN1, OCTN2 and OCTN3 have been identified. OCTN1 & 2 seems to be involved in carnitine and acetylcarnitine transport in spermatozoa (Kobayashi et al., 2007). OCTN2 has been shown to be present in brain capillary endothelial cells where it transports carnitine and acetylcarnitine into the brain (Kido et al, 2001). While, OCTN1 appears to be a more general cation transporter for a number of cationic drugs such as desipramine, dimethylamiloride, cimetidine, procainamide and is localised in rat kidney and brain (Wu et al., 2000). All three OCTN's have been detected in murine brain (Lamhonwah et al., 2007).

Recently OCT-1, 2 & 3 have been stably transfected into the human kidney epithelial cell line (HEK) and the transport of paraquat into these cells examined (Chen et al., 2007). No uptake was seen with OCT3 and very little with OCT1; the major transporter appeared to be OCT2. They demonstrated for the first time that the

di-cation paraquat is a substrate for OCT 2 with a K_m of $114 \pm 23 \mu M$ and a V_{max} of $174 \pm 37 \text{ pmol/min/mg protein}$. Consistent with these findings they showed that paraquat was more cytotoxic to HEK cells containing OCT2 ($EC_{50} 23 \mu M$) compared to those with a mock transporter ($EC_{50} 417 \mu M$) i.e. 18-times more sensitive.

In the kidney, OCTs are located on the basolateral membrane transporting chemicals into renal tubule cells, in order for chemicals to undergo active secretion by the kidney they also need to undergo transport at the brush border membrane into the tubule lumen. This phenomenon has been observed and widely reported but the transporters involved were only relatively recently identified by Otsuka et al (2005) who reported that a human transport protein MATE1 (multidrug and toxin extrusion 1) mediated the final excretion step for organic cations. This was followed by the identification of MATE2 which appears to be present primarily in the kidney (Masuda et al., 2006). MATE 1 is present, primarily but not exclusively, in the kidney of rats, humans, rabbits and mice (Hiasa et al., 2006; Zhang et al., 2007; Kajiwara et al., 2007). Chen et al., (2007) expressed MATE1 in HEK cells and studied the transport of paraquat and showed uptake that obeyed saturation kinetics with a K_m of $212 \pm 19 \mu M$ and a V_{max} of $289 \pm 46 \text{ pmol/min/mg protein}$. As with the OCT2 cells they demonstrated that paraquat was more toxic to those cells containing MATE1 compared to those without it ($EC_{50} 125 \mu M$ versus $717 \mu M$). This seminal paper thus showed that paraquat can enter cells via OCT2 and leave via MATE1.

What relevance does this have to the brain? The brain is a protected organ such that many foreign chemicals are excluded by the blood-brain barrier, which basically comprised of endothelial cells lining the blood vessels having very tight junction. However, the brain needs substrates such as glucose, amino acids and neurotransmitters to function and these enter the brain by a range of transport systems located on capillary endothelial cells. There are currently 17 members of the human SLC6 transporter family involved in the transport of amino acids, monoamines, GABA, osmolytes and creatine plus 10 members of the OCT and OAT (organic anion transporters). Thus foreign compounds are generally excluded from the brain unless: they mimic a natural substrate e.g. methylmercury-cysteine mimics methionine; are lipid soluble and can enter by diffusion or unless they enter in those regions of the brain where the blood brain barrier is leaky. These include the olfactory region, circumventricular region, area postrema and hypothalamus.

Paraquat can enter cells via OCT2 and OCT2 has been detected in the choroid plexus of the rat (Sweet et al., 2001). OCT2 & 3 are expressed in rat brain predominantly in regions located at the brain CSF fluid border, in the leptomeninges such as the choroid plexus and surrounding the ventricles. OCT3 is also located to these regions but also extends to monoaminergic pathways such as raphe nuclei, striatum and thalamus. A low level of expression was also detected in the granule cell layer of the cerebellum and in the granule layer and pyramidal cells of the hippocampus in the CA1-3 regions (Amphoux et al., 2006). OCT2 has also been detected in human brain, northern blotting showing it present in the thalamus, hippocampus, nucleus caudatus but below the limit of detection in the substantia nigra (Busch et al., 1998). In situ hybridisation with hOCT2-specific cRNA showed hybridisation in pyramidal cells of the human cerebral cortex and hippocampus (Busch et al., 1998). Functional studies indicate that in the brain OCT2 and OCT3 from rat and human appear to have a role in monoamine transport with a preference for histamine and adrenaline (Busch et al., 1998; Amphoux et al., 2006).

In summary, little information is available on OCT in the brain; however it is an area of active research and with time more detailed information will become

available. Paraquat can enter cells via an OCT2 transporter or in the case of the CSF – blood barrier leave the brain via this transporter. At the present time it is unclear how this may be linked to damage to the substantia nigra of the C57Bl6 mouse. OCT2 is present in rat, mouse and human kidney and rat and human brain but studies in mouse brain have not been reported. OCT3 has been detected in the striatum of rat brain, however Chen et al., (2007) reported that paraquat is not a substrate for this transporter. Thus the picture is far from clear. In addition, just to confuse the issue, Shimizu et al., 2001 and McCormack et al., 2003 showed that large doses of substrates for the neutral amino acid system (e.g. L-valine) could prevent paraquat entry and hence neuronal cell loss in the substantia nigra of C57Bl/6 mice, indicating a role for this transport system. Whether paraquat does indeed enter the brain via the neutral amino acid transport system is unclear and in time another explanation for this observation may be found. What is now beyond doubt is that paraquat can enter dopaminergic neurons in the substantia nigra of C57Bl6 mice in sufficient concentration to cause cell death. OCT's appear to be fairly well conserved across mammalian species such that paraquat would be expected to undergo transport in a similar manner across species. The basis for the particular sensitivity of the C57Bl6 mouse, assuming it is a transport issue, may be due to a number of factors such as an absence of MATE1 or 2 to facilitate removal or a lower level of antioxidants to protect against the injury produced.

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