Liver transplantation in PNPO deficiency: management challenges and biological lessons

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Background

Pyridox(amine)ine S’ Phosphate Oxidase (PNPO) deficiency is an autosomal recessive disorder of vitamin B6 metabolism caused by mutations in the PNPO gene (Figure 1). It usually presents with refractory neonatal seizures responsive to treatment with pyridoxal 5’ phosphate (PLP).

A 15 y.o. boy with neonatal onset PNPO deficiency (heterozygous mutations in PNPO/PLP dependency) complicated by long-standing cirrhosis developed hepatocellular carcinoma without extra-hepatic disease. It was decided that the optimal treatment for this was liver transplantation. Prior to transplantation he was neurologically and cognitively normal and seizures were well controlled on 50mg/kg/day PLP.

Liver transplantation has not previously been performed in PNPO deficiency and provided considerable management challenges. Liver transplantation is a unique biological model to understand PNPO deficiency as the transplanted liver has normal PNPO function whereas neurones continue to lack PNPO.

Figure 1: Metabolism of PLP and other B6 vitamers

Methods

Continuous EEG monitoring was initiated pre-operatively and continued to aid with identifying cerebral PLP deficiency. Doses of PLP were titrated to avoid PLP deficiency (symptoms, EEG findings, seizures) and to minimise biochemical and clinical evidence of hepatic dysfunction (changes in Liver Function Tests [LFT], hepatic synthetic capacity and alpha fetoprotein [AFP]). B6 vitamers (pyridoxine [PN], pyridoxamine [PM], pyridoxal [PL] and phosphorylated forms [PNP, PMP, PLP]) were assayed at times of deficiency (symptoms and/or EEG) and for pharmacokinetics.

The study was approved by the Sydney Children’s Hospital Network institutional ethics committee (Project No. CCR2020/29).
Results

1. Liver transplant and PLP requirements

PRE-TRANSPLANT - Oral PLP dosage: The patient was taking PLP 50mg/kg/day in 4 hourly doses with a larger night-time dose and no overnight doses.

POST-TRANSPLANT – Intravenous (IV) PLP infusion: During and post-liver transplantation, PLP treatment was provided parenterally by infusion. PLP was initially infused at a rate of 1.1 mg/kg/hr (26.4 mg/kg/day) and was able to be weaned to 0.35 mg/kg/hr (8.5 mg/kg/day) before evidence of PLP deficiency emerged.

POST-TRANSPLANT - Intravenous PLP bolus doses: In order to transition from PLP infusion and to provide a more physiological pattern of delivery of PLP, IV 4 hourly bolus doses were instituted at a dose of 13 mg/kg/day and weaned to 7.8 mg/kg/day before evidence of deficiency emerged.

POST-TRANSPLANT - Oral PLP dosage: Transition from intravenous to oral dosing of PLP proved to be extremely difficult. Rapid changes in oral dosage resulted in episodes of deficiency which did not occur subsequently at the same dose if the dose was incrementally decreased. Shorter dosing intervals (3rd hourly) allowed lower doses to be given. Overall it took 22 months to establish the patient on a dose of PLP which minimised hepatotoxicity and gastro-intestinal side effects (nausea, vomiting) and avoided episodes of deficiency. The final treatment dose was 24 mg/kg/day given in three hourly doses (other than overnight). This dose of PLP left very little leeway should a dose of PLP be delayed or omitted.

2. PLP showed dose dependent hepatotoxicity post transplant

EXTREMELY HIGH DOSE ORAL PLP CAUSED ACUTE HEPATIC INFLAMMATION: Two weeks post transplant, oral PLP was introduced with IV PLP at the pre-transplant dose of 55 mg/kg/day and then IV PLP was gradually withdrawn. This was associated with recurrent episodes of deficiency and so the dose was rapidly escalated to 100mg/kg/day. Following this, the patient became nauseous and there was a severe deterioration in his liver function (Figure 2) which resolved with re-institution of IV PLP.

Figure 2: LFT deterioration on PLP 100mg/kg/day

DOSE DEPENDENT ORAL PLP HEPATOTOXICITY: The transitions from IV PLP to oral PLP were associated with repeated deteriorations in hepatic function (predominantly elevations of AST/ALT and increases in alpha fetoprotein [αFP]) often associated with anorexia which reliably improved with reductions in oral PLP dosage and resolved with the re-institution of IV PLP treatment.

Figure 2: This figure shows the severe deterioration in hepatic function that occurred with a rapid transition from IV to high dose oral PLP. IV PLP was recommenced on 15/9/2018 and oral PLP was ceased. Extremely elevated PL levels were measured at the time of the acute deterioration.

PL levels: 32200 nmol/l (5/9/18) prior to the episode of hepatotoxicity, and 93020 (13/9/18), 63040 nmol/l (14/9/18) [N – 5-18 nmol/l].
PLP levels: 25680 nmol/l (5/9/18) prior to the episode of hepatotoxicity, 1020 ((13/9/18) and 3180 nmol/l (14/9/18) [N – 46-321 nmol/l].
Liver biopsy confirmed this was not due to rejection.
Results

3. B6 vitamer levels

Figure 4: B6 vitamer profiles: IV vs oral

4. B6 vitamer levels associated with deficiency episodes

B6 vitamer levels were measured during episodes of symptomatic and/or EEG PLP deficiency. Results of blood collections performed during episodes where there was definite evidence of encephalopathy (clinical symptoms and/or seizures + EEG evidence of neuronal PLP deficiency) are presented below.

B6 vitamer levels during episodes of encephalopathy while on enteral PLP therapy

Four episodes of deficiency occurred during which B6 vitamer levels were collected.

Table 1: B6 vitamer levels during episodes of encephalopathy on enteral PLP

<table>
<thead>
<tr>
<th>Sample</th>
<th>PL nmol/l (N 5-18)</th>
<th>PLP nmol/l (N 46-321)</th>
<th>PM nmol/l (N 0-0)</th>
<th>PN nmol/l (N 0-0.6)</th>
<th>PA nmol/l (N 16-139)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>790</td>
<td>384</td>
<td>182</td>
<td>226</td>
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<td>2225</td>
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<td>224</td>
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<td>915</td>
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<td>310</td>
<td>2359</td>
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<td>1223</td>
<td>732</td>
<td>230</td>
<td>533</td>
<td>2364</td>
</tr>
<tr>
<td>Mean</td>
<td>1289</td>
<td>720</td>
<td>379</td>
<td>1514</td>
<td>2401</td>
</tr>
</tbody>
</table>

Table 2: B6 vitamer levels during episodes of encephalopathy on IV PLP

<table>
<thead>
<tr>
<th>Sample</th>
<th>PL nmol/l (N 5-18)</th>
<th>PLP nmol/l (N 46-321)</th>
<th>PM nmol/l (N 0-0)</th>
<th>PN nmol/l (N 0-0.6)</th>
<th>PA nmol/l (N 16-139)</th>
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</thead>
<tbody>
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<td>20785</td>
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<td>2</td>
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<td>17772</td>
<td>4941</td>
<td>102</td>
<td>1998</td>
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<tr>
<td>Mean</td>
<td>2522</td>
<td>19278</td>
<td>5008</td>
<td>111.5</td>
<td>2185</td>
</tr>
</tbody>
</table>

Footnote Table 2: These episodes occurred while on a combination of therapy with IV PLP 5.8 mg/kg/day and oral PM 8mg/kg/day (hence the elevated levels of PM).

All episodes of encephalopathy occurred with PL levels below 2800 nmol/l although levels below this were frequently tolerated without encephalopathy especially if PLP levels were high (e.g. while on IV PLP). PL levels below 1000 nmol/l were more strongly associated with encephalopathy. As can be seen episodes of encephalopathy could occur with very high levels of PLP.
Results:

5. Pharmacokinetics of oral and intranasal PLP

Figure 5: Pharmacokinetics of oral PLP

The chart shows levels of B6 vitamers after a single dose of PLP was given orally (10:00 on the chart). Prior to the dose the patient was being treated with IV PLP. The peak PL level was not reached until 2.5 hours post dose. PLP levels continued to fall despite the oral dose. PA is noted to rise more rapidly than PL suggesting a proportion of the oral dose rapidly undergoes hepatic conversion. Similar profiles were noted with other oral doses.

Figure 6: Pharmacokinetics of intranasal dosing of PLP

The chart shows levels of PL and PLP after a single intranasal dose of the IV solution of PLP was given intranasally at 10:00. PL rose from 2092 nmol/l to a peak of 7945 nmol/l within 18 minutes of the dose. Moreover PLP was noted to rise (in contrast to oral dosing).

6. Treatment of episodes of PLP deficiency: rate of response

EEG monitoring

EEG proved invaluable in differentiating between episodes of PLP deficiency and other causes of anxiety/distress in the post-transplant period. Epileptiform discharges were noted only at times of deficiency.

Intravenous administration of PLP (usual dose 30mg ~ 0.5 mg/kg) was associated with almost immediate resolution of epileptiform discharges whereas intranasal and oral treatment showed delayed resolution. Clinically and electrophysiologically intranasal PLP (60mg) proved faster in reversing episodes of encephalopathy than oral PLP and so this was subsequently added as a rescue medication to oral PLP.

7. Treatment with pyridoxamine (PM) and pyridoxine (PN)

In an attempt to limit the hepatotoxicity associated with high doses of PLP, oral PM (12mg/kg/day) and intravenous PN were added to his treatment. These treatments made no difference to requirements for PLP and were associated with episodes of encephalopathy at doses of PLP that otherwise would have been tolerated.
Liver transplantation did not cure PNPO dependent epilepsy.

Post liver transplant, impairments in neuronal recycling of PLP necessitated an ongoing requirement for supraphysiological doses of PLP. Prior to transplant the patient was receiving 50 mg/kg/day PLP and post transplant the dosage was 24 mg/kg/day. It seems likely that the lower requirement for PLP post-transplant resulted from more aggressive titration of PLP dosage rather than a decrease in PLP requirements. Neither oral pyridoxamine nor pyridoxine decreased dosage requirements for PLP.

IV PLP requirements were approximately one third of oral PLP requirements.

Prolonged treatment with PLP proved very effective in preventing episodes of encephalopathy associated with PLP deficiency. PLP associated hepatotoxicity was not seen while on IV PLP (despite considerably higher PL and PLP levels). However, the use of IV PLP was associated with a very difficult and prolonged transition from IV to oral PLP (approximately 6 months).

Oral PLP showed dose dependent hepatotoxicity post liver transplant.

Acute hepatic inflammation was associated with the use of extremely high doses of oral PLP (100 mg/kg/day). This resolved with re-institution of IV PLP. Hepatic inflammation showed consistent improvement on weaning oral PLP doses.

Blood PL levels below 2800 nmol/l were associated with an increased risk of encephalopathy.

PL levels below 1000 nmol/l were strongly associated with encephalopathy. PLP levels were only weakly associated with episodes of encephalopathy.

Off-label use of intra-nasal PLP (60mg) proved effective in rapidly treating episodes of encephalopathy.

Intranasal PLP given at times of encephalopathy associated with PLP deficiency resulted in faster resolution of episodes than oral PLP. Pharmacokinetic data supported this and showed more rapid rises in PL after intra-nasal doses than after oral doses. The rise in PLP post intranasal dosing (not seen with oral dosing) suggests that PLP may have been directly absorbed across the nasal mucosa.

Conclusions

Study limitations

While considerable data was obtained on this patient in order to manage him post liver transplant, it is unclear whether this data generalises to patients with different genetic mutations causing PNPO deficiency. It is not clear how the pharmacokinetic data applies to people with PNPO deficiency who have not had liver transplantation.

Acknowledgements:

The patient and his parents contributed considerably to this study. The patient’s suggestion about trying shorter dosage intervals proved critical to permitting minimal dosing with PLP. His parent’s help in sorting pharmacokinetic data was invaluable in this analysis.