Children’s toxicology from bench to bed - Liver Injury (1):
Drug-induced metabolic disturbance
- Toxicity of 5-FU for pyrimidine metabolic disorders and
pivalic acid for carnitine metabolism -

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ABSTRACT — Congenital disorders of metabolism show a wide spectrum of symptoms as a conse-
quence of impairment of a certain metabolic pathway by mutated enzymes resulting in abnormal accumu-
lation of enzyme substrates, deficiency of expected products, and abnormal burden to collateral metabolic
pathways, etc. However, in some occasions, depending on which pathway up to what degree of distur-
bance, it can be asymptomatic until a certain kind of burden is placed on to the patient. Enzyme deficien-
cy involved in pyrimidine degradation, such as Dihydropyrimidine dehydrogenase (DPD) and Dihydro-
pyrimidinase (DHP), has been reported with convulsion or autism as symptoms, but many asymptomatic
cases are also reported. However, when the patients are treated with 5-fluorouracil, a pyrimidine ana-
logue anticaner drug, lethal side-effects can be seen even in asymptomatic patients. Some oral cephem
antibiotics have pivalic acid side chain to increase absorption rate at intestine. These antibiotics degrade
into active antibiotics and pivalic acid at the intestinal wall. This pivalic acid is carnitine-conjugated and
excreted into urine. Carnitine acts as a carrier of long chain fatty acid to mitochondria and to beta-oxidation,
thus an important molecule for energy production by beta-oxidation and maintenance of mitochon-
drial function. Because of this, long term administration of such antibiotics could induce depletion of car-
nitine from the body and lead to low ketotic hypoglycemia, convulsion and consciousness disturbance.
This paper reports some possible serious side effects closely linked to drug metabolism.

Key words: Dihydropyrimidine dehydrogenase, Dihydropyrimidinase, Pivalic acid, Carnitine

Toxicity of 5-FU for pyrimidine metabolic disorders

Pyrimidines, such as uracil and thymine are the impor-
tant components of DNA, RNA and other parts of the
body. In the pathway of their degradation, three enzymes,
dihydropyrimidine dehydrogenase (DPD), dihydropy-
rimidinase (DHP), and \( \beta \)-ureidopropionase are involved
(Fig. 1). And congenital abnormalities for each enzyme
are known. DPD deficiency, the most important among
them, is reported in Europe and the United States with
major symptoms of convulsion and psychomotor retar-
dation. A whole variety of symptoms of this disease are
including growth retardation, autism, dysmorphism and
even asymptomatic cases (Webster et al., 2001).

We have developed a simplified assay method for urine
uracil and hydroxyuracil by a short-step pre-treatment to
a column switching high-performance liquid chromatog-
rphy (HPLC) (Fig. 2) (Ohba et al., 1995). Using this
assay, we conducted a mass-screening of Japanese pop-
ulation for DPD deficiency and DHP deficiency which is
an enzyme defect of the next step of the metabolic path-
way.

Subjects are the 34,200 newborns presented their blot-
ted urine for neuroblastoma mass screening and consented
to this survey. Blotted two filter papers of 5mm in diame-
ter were extracted with 5% methanol, and subjected to the
column switching HPLC method. As a result, three case
of asymptomatic DHP deficiency was identified. As men-
tioned above, Majority of Western case reports are symp-
tomatic, with occasional neuronal symptoms such as con-
vulsion and mental retardation. Our data indicates that all
Japanese cases are asymptomatic, with a frequency of 1
to 11,000 (Imaeda et al., 2000).

5-Fluorouracil (5-FU) is a representative of pyrimi-
dine analogue anticancer drugs, and used against various malignant neoplasias including digestive tract cancers, such as gastric and colon carcinomas. This 5-FU, a uracil derivative, shares the metabolic pathway with uracil (Maeda et al., 1999). Consequently, when 5-FU is administered to the above-mentioned DPD deficiency or DHP deficiency patients, due to insufficient degradation, abnormally high retention of 5-FU is induced with lethal side effects (Webster et al., 2001). It is noted that DPD heterozygous deficiency, who is the gene carrier, suffers from abnormal 5-FU toxicity (Webster et al., 2001). Recently, deficiency of the third enzyme of the pyrimidine metabolic pathway, the β-ureidopropionase deficiency is also reported (Van Kuilenburg et al., 2001). This patient may

Fig. 1. Pyrimidine degradation pathway.
exhibit abnormal toxicity to 5-FU treatment, and close follow up is needed.

**Pivalic acid for carnitine metabolism**

Pivalic acid, a carboxylic acid with (CH₃)₃CCOOH in its structure, is well known as a component of produgs by its ability to highly increase drug absorbance in the gastrointestinal tract when ester bound to the drug molecule. Fig. 3 shows the structures of the pivalic acid-bound antibiotics widely used in pediatrics, otolaryngol-
ogy clinics and others. These antibiotics are available in dried syrup formulations for small children and are recommended for the treatment of penicillin-resistant-Streptococcus pneumoniae (PRSP) and other resistant bacterial infections. This prodrug is degraded into active antibiotics and pivalic acid by the intestinal epithelial cells, and the pivalic acid is carnitine-conjugated and excreted into urine. Long-term administration of such antibiotics is known to induce hypocarnitinemia (Melegh et al., 1987).

We experienced a case of a one-year-old male who...
was administered with several different antibiotics for six months in a row for recurrent otitis media and recurrent upper respiratory tract inflammation. The patient showed tremor of the hands and feet in the morning, propagated to generalized convulsion and brought to an emergency outpatient. On consultation, blood glucose was 18 ml/dl but blood ketone body was only slightly elevated, and fatty acid oxidation defect was suspected. Blood ketone body can be produced as acetyl-CoA metabolite. However, in the starving situation of hypoglycemia causing generalized convulsion, fatty acid is mobilized by carnitine into mitochondria, and beta-oxidized to produce energy. Acetyl-CoA produced by this beta-oxidation is normally metabolized to ketone body to induce ketosis. Hypoglycemia without ketone body production leads to an assumption that the fatty acid oxidation is in failure. This case showed 3.2 micro mol/l of blood free carnitine and 4.1 micro mol/l of total carnitine, which are about 1/10 of normal levels. This was considered as a result of prolonged administration of pivalic acid administration. Again, pivalic acid is almost exclusively excreted as carnitine conjugate, and almost none by other conjugates or free molecule. Therefore the amount of loss of carnitine from the body is almost equal to the amount of administered pivalic acid. The total estimate of the administered pivalic acid in this case was about 21 mmol, which was twice as much as estimated preserved amount of carnitine of a 10 kg body weight child; 11 mmol. In addition to these findings, our HPLC-tandem-mass spectrometer system (Maeda et al., 2007) (Fig. 4) which can separate and determine acyl-carnitine isomers revealed that in spite of extremely low blood free carnitine, same amount of pivaloyl-carnitine was detected, indicating that the binding of pivalic acid to carnitine is extremely strong. After a few similar cases were reported, warning for decrease in carnitine has been added to the Drug Package Inserts. It is essential for every clinician to pay attention to possible side-effects in relation to fatty acid metabolism, especially when new drugs of similar property are released to the market.

![Chromatogram of standard acylcarnitine mixture using HPLC/MS-MS method.](image-url)
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REFERENCES


