

OBJECTIVES

- To test the hypothesis that valproic acid sodium salt, diclofenac sodium salt, ketoprofen, PFOS, PFOA and PFNA would be incorporated into the α -ketoacid dehydrogenase complex (PDC-E2) *in vitro*.
- To test if there is an effect of the concentration of chemicals and incorporation into PDC-E2 *in vitro*.

INTRODUCTION

- Primary biliary cholangitis (PBC) is an auto-immune liver disease triggered by lipoid acid-mimicking xenobiotics, leading to production of high titer of antimitochondrial autoantibodies recognising E2 subunit of pyruvate dehydrogenase (PDC-E2). (1)
- PDC-E2 enzyme, found within the inner mitochondrial matrix, has an N-terminal domain consisting of lipoid acid that forms an antigen (2).
- Due to environmental exposure, genetically susceptible patients develop autoimmunity, i.e. their immune system loses self-tolerance, thus fails to recognise self-molecules, which can lead to cellular injury (3).
- Previous studies have demonstrated that loss of tolerance to PDC-E2 is caused by xenobiotic modification of native lipoid acid.

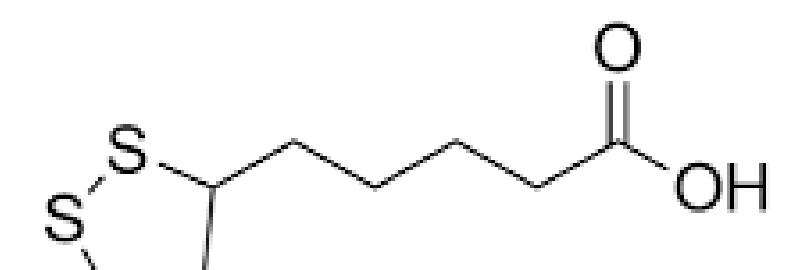


Figure 1. Lipoic acid

METHODS

Table 1. Composition of samples used in Western Blotting

Tube	Chemical	Volume of PDC-E2-ILD (μ l)	4mM ATP+GTP (μ l)	LAE/LT (μ l)	20 mM Tris-HCl (pH 7.5) buffer (μ l)
A	none	2.5	0	0	47
B	none	2.5	0.5	0	46.5
C	none	2.5	0.5	1	45.5
D	lipoic acid	2.5	0.5	1	45.5
E	BMI	2.5	0.5	1	45.5
F	MBO	2.5	0.5	1	45.5
G	PFDA	2.5	0.5	1	45.5
H	PFNA	2.5	0.5	1	45.5
I	diclofenac	2.5	0.5	1	45.5
J	valproic acid	2.5	0.5	1	45.5
K	ketoprofen	2.5	0.5	1	45.5
L	PFOS	2.5	0.5	1	45.5

0.5 μ l of the chemical was introduced in each tube to make up to 50 μ l reaction mixture. Abbreviations: PDC-E2, dihydrolipoamide acetyl-transferase; ILD, inner lipoyl domain; LAE, lipoate activating enzyme; LT, lipoyl-AMP(GMP):N-lysine lipoyl transferase

WESTERN BLOTTING

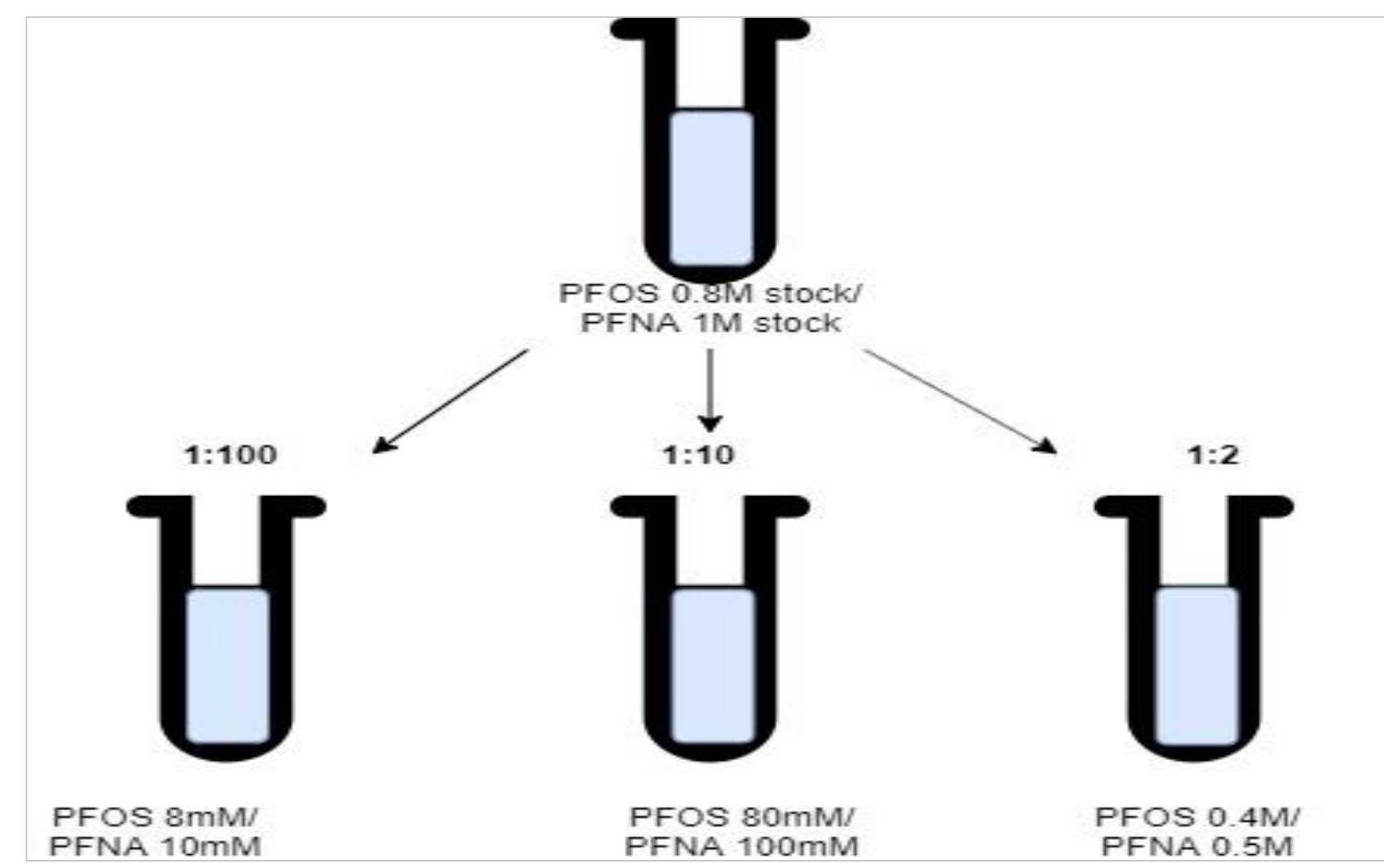


Figure 2. Simple dilution of PFOS and PFNA stock concentration

Table 2. Concentrations of the chemicals used in gel electrophoresis (see Figure 4)

Tube	Chemical	Concentration
A1	PFOS	8mM
B1	PFOS	80mM
C1	PFOS	0.4M
D1	PFNA	10mM
E1	PFNA	100mM
F1	PFNA	0.5M

RESULTS

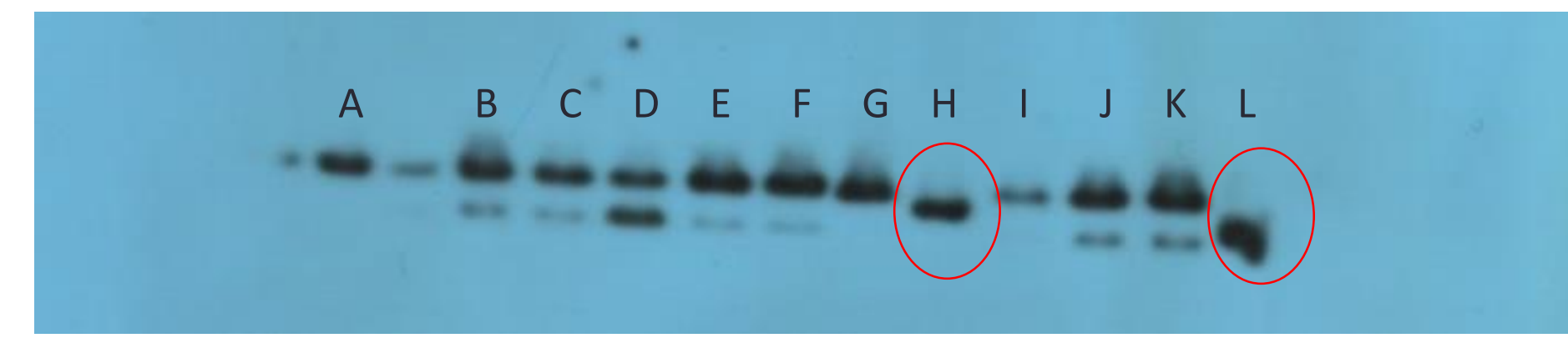


Figure 3. Xenobiotic incorporation into PDC-E2 complex

- A-C lanes: negative control; visible upper (unlipoylated) band; no or minimal lipoylation of PDC-E2.
- D lane: positive control; presence of a lower (lipoylated) band
- E-F lanes: presence of lipoylated band indicating minimal incorporation
- G lane: unlipoylated PDC-E2
- H lane: **a visible shift in a band compared with negative control indicating lipoylation**
- I lane: upper band demonstrating no lipoylation of PDC-E2 complex
- J-K lanes: unlipoylated band is thicker than a lipoylated band
- L lane: **a visible shift in a band compared with negative control indicating full lipoylation**

CONCLUSION

- It was found that only PFNA and PFOS were incorporated into the PDC-E2-ILD complex.
- Further experiments indicated that concentrations of 10mM, 100mM and 0.5M of PFNA were sufficient for incorporation.
- 2/3 negative controls showed minimal lipoylation due to recombinant proteins LAE, LT and PDC-E2-ILD having passenger *E. Coli* lipoylation enzymes.
- Further methods, such as Mass spectrometry is needed to validate the PDC-E2-ILD reaction products.

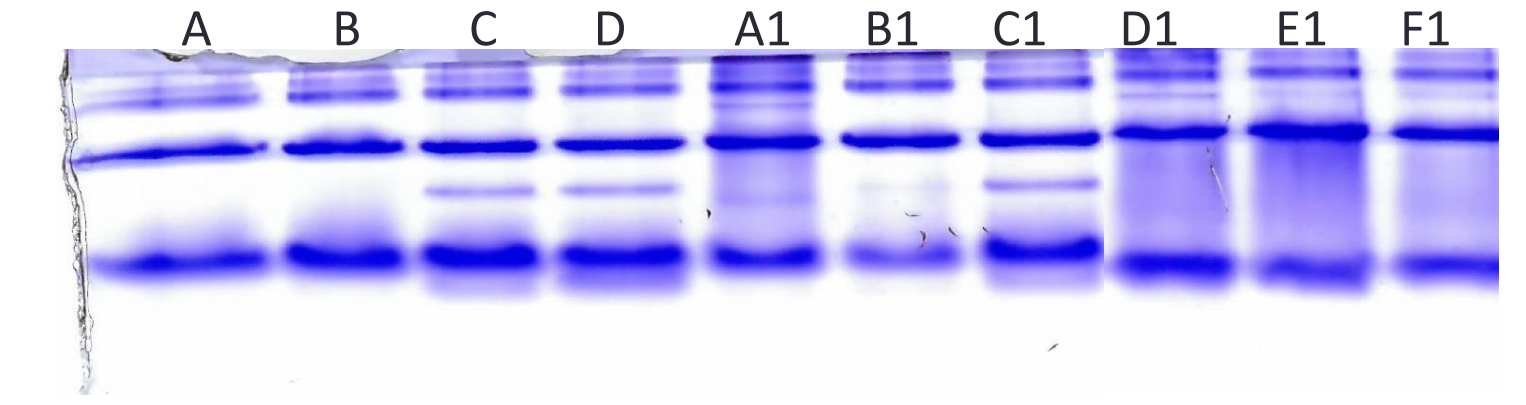


Figure 4. Effect of concentrations on xenobiotic incorporation into PDC-E2 complex

- A-C lanes: negative control; visible upper (unlipoylated) band; no or minimal lipoylation of PDC-E2.
- D lane: positive control; presence of a lower (lipoylated) band
- A1-B1 lanes: no lipoylation of PDC-E2
- C1 lane: a visible lower band indicating lipoylation
- D1-F1 lanes: **a visible shift in a band indicating full lipoylation**

REFERENCES

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