Analysis of real-world data and a mouse model indicates that pirfenidone causes pellagra

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Shareable abstract (@ERSpublications)
Some adverse effects of pirfenidone (PFD) appear to resemble pellagra but this is the first report to show this phenomenon. Verification is required but side-effects in patients with idiopathic pulmonary fibrosis on PFD may be relieved by niacin. https://bit.ly/3b1kvuR

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Abstract

Background Pirfenidone (PFD) is widely used in patients with idiopathic pulmonary fibrosis (IPF) and its adverse effects, such as nausea and photosensitivity, are well known. Many patients with IPF have reduced doses or even cessation of PFD because of its side-effects. No solutions have been found for these side-effects because the current mechanistic insights are insufficient.

Methods Using the results of real-world data analysis from the US Food and Drug Administration Adverse Events Reporting System, we hypothesised that PFD-related symptoms may be similar to pellagra. Reverse translational experiments using female BALB/c mice were performed to validate and estimate this hypothesis. Niacin and its metabolite responses were compared between patients with IPF treated with PFD and those treated without PFD.

Results The pellagra hypothesis was translated from real-world data analysis. Pharmacological and comprehensive genetic investigations showed that PFD caused pellagra-related nausea and photosensitivity in a mouse model, which may have been mediated by the actions of nicotinamide *N*-methyltransferase (NNMT). Higher NNMT substrate responses were observed in urine from patients and mice with PFD than in those without PFD.

Conclusions PFD may cause pellagra or pellagra-like symptoms such as photosensitivity. Further studies are required to investigate whether niacin prevents pellagra-like symptoms caused by PFD in patients with IPF.

Introduction

The efficacy and safety of pirfenidone (PFD) have been determined by phase III clinical trials of Japanese patients with idiopathic pulmonary fibrosis (IPF) [1–4]. Some adverse effects, such as photosensitivity, gastrointestinal events (nausea and vomiting), fatigue, decreased appetite and diarrhoea, were observed in these trials. However, the cause of these adverse effects is unknown. We hypothesise that a relatively large amount of PFD acts as a modulator in the metabolic flow of tryptophan–niacin and causes pellagra-like symptoms because the structure of PFD resembles that of niacin and its metabolites. An interesting approach to attenuate adverse effects is the use of a respirable powder formulation of PFD, which is used to lower the dose [5]. Pellagra is defined as severe niacin deficiency characterised by the "three or four Ds" of dermatitis, diarrhoea and dementia or, if left untreated, death [6–8]. Deficient intake of niacin or tryptophan from dietary sources (primary pellagra) and defective use of these nutrients (secondary pellagra) are thought to be types of this disease [9]. Occasionally, secondary pellagra caused by isoniazid (INH), which is an anti-tuberculosis drug used globally, causes pellagra-related photosensitivity and nausea [6–11].

Various real-world data-based methods have attracted attention in pharmacovigilance and epidemiological studies, and these were developed to translate real-world data to a hypothesis, such as unexpected associations between drugs and adverse effects. In these fields, sequence symmetry analysis and disproportionality analysis are widely used because of their moderate sensitivity and high specificity [12]. These methodologies enable detection of unknown signals caused by available medicines. In accordance with this concept (figure 1), some investigators have successfully found drug "A" that may mitigate the risk of adverse effects associated with the use of drug "A" *via* the analysis of real-world data from the US Food and Drug Administration Adverse Events Reporting System (FAERS). This is the largest worldwide database of self-reports of adverse drug events. FAERS is freely available to the public and is considered to be a drug-induced symptom database of humans, containing many cases of polypharmacy [13–16]. FAERS enabled us to find confounding factors as drug "A" which may affect the occurrence of PFD-induced symptoms.

In this study, using real-world data analysis, we hypothesised that some side-effects caused by PFD are similar to those caused by pellagra. We also carried out reverse translational research using the proper mouse models [11, 17, 18] to test this hypothesis.

Methods

Real-world data analysis

Briefly, the adverse event risk was evaluated by calculating the reported odds ratios with 95% confidence intervals in accordance with previously described methods (see supplementary material) [13–15].

Experimental animals and designs

Briefly, data from experiments are reported in accordance with the Animal Research: Reporting of *In Vivo* Experiments guidelines [19]. Nausea was evaluated quantitatively by pica behaviour in accordance with previous studies (see supplementary material) [11, 18].

Liquid chromatography-mass spectrometry

Ear levels of prostaglandin E2 (PGE2), epoxyeicosatrienoic acids (EETs) and dihydroxyeicosatrienoic acids (DHETs) were investigated in accordance with previous studies [20–22]. Urine levels of niacin and its metabolites were investigated in accordance with a previous study [11].

Subjects and sample collection

Urine from patients diagnosed with idiopathic interstitial pneumonia at Sapporo Medical University Hospital (Sapporo, Japan) (table 1) and control subjects at the Shionogi and Okayama Biobank were used to analyse niacin and its metabolites.

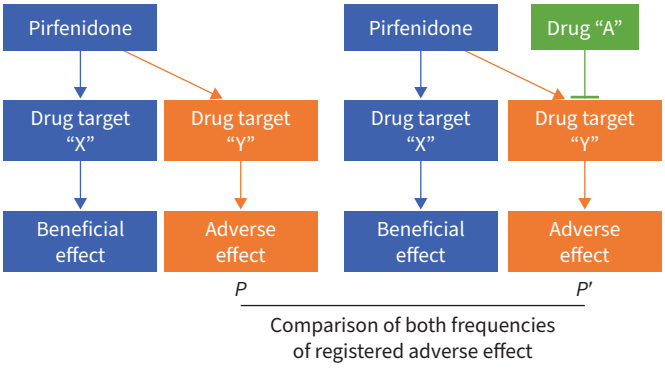

FIGURE 1 Concept of the real-world data analysis. The analytical concept to discover drug "A" is shown. The frequency of registered adverse effects in patients with pirfenidone (PFD) was compared with that in patients with PFD treated with drug "A". When $P>P'$ was observed, drug "A" played a role in the development of adverse effects caused by PFD. A total of 11 428 031 reports from the US Food and Drug Administration Adverse Events Reporting System (FAERS) (from Quarter 1 2004 to Quarter 4 2019) were analysed. After identification of the name, 3568 types of medicines were investigated. The frequencies (%) of patients with and without PFD who suffered from photosensitivity were compared. Unknown drug–drug interactions against photosensitivity caused by PFD (figure 2a and b) and nausea caused by PFD or nintedanib (figure 2c and d) were evaluated in accordance with the Methods.

TABLE 1 Baseline characteristics of the study subjects

	Without pirfenidone	With pirfenidone	p-value
Subjects	10	10	
Age (years)	72 (67–85)	75.5 (67–88)	0.1527
Male/female	8/2	8/2	
BMI ($kg \cdot m^{-2}$)	26.2 (25.2–27.6)	24.0 (21.6–25.8)	0.1926
Brinkman index	800 (75–1743)	900 (39.8–1490)	0.6855
VC (L)	2.31 (2.07–3.09)	2.61 (2.19–2.91)	0.588
VC (% pred)	76.2 (68.3–92.2)	80.9 (70.7–92.5)	0.4608
FVC (L)	2.26 (1.97–3.08)	2.64 (2.16–2.84)	0.5236
FEV_1 (L)	1.93 (1.72–2.46)	2.34 (1.82–2.52)	0.3674
FEV_1 (% pred)	84.1 (77.2–89.3)	85.8 (79.0–91.8)	0.7371
D_{LCO} ($mL \cdot min^{-1} \cdot mmHg^{-1}$)	12.9 (10.8–14.8)	10.2 (9.3–13.3)	0.3838
D_{LCO} (% pred)	59.8 (53.5–71.3)	43.6 (36.0–55.3)	0.0369
P_{aO_2} (mmHg)	89.1 (81.6–93.4)	82.7 (77.6–88.3)	0.2635
P_{aCO_2} (mmHg)	38.9 (36.8–41.4)	40.5 (39.4–43.7)	0.1267
S_{pO_2min} of 6MWT (%)	92 (87.5–94)	90 (87.5–95.3)	0.861
6MWD (m)	430 (320–453)	430 (358–485)	0.6767
SP-A ($ng \cdot mL^{-1}$)	88.1 (46.6–111.7)	70.7 (45.3–114.5)	0.8306
SP-D ($ng \cdot mL^{-1}$)	323 (192.23–355.8)	204.5 (114–314.5)	0.2347
KL-6 ($U \cdot mL^{-1}$)	1100 (621.8–2218.8)	1185 (803.8–1690)	0.3867
LDH ($IU \cdot L^{-1}$)	255.5 (224.8–312)	245 (223.3–300.3)	0.8981

Data are presented as n or median (interquartile range), unless otherwise stated. BMI: body mass index: VC: vital capacity; FVC: forced vital capacity; FEV_1: forced expiratory volume in 1 s; D_{LCO}: diffusing capacity of the lung for carbon monoxide; P_{aO_2}: arterial oxygen tension; P_{aCO_2}: arterial carbon dioxide tension; S_{pO_2}: oxygen saturation measured by pulse oximetry; 6MWT: 6-min walk test; 6MWD: 6-min walk distance; SP-A: surfactant protein A; SP-D: surfactant protein D; KL-6: Krebs von den Lungen-6; LDH: lactate dehydrogenase.

This retrospective study was approved by the hospital's ethics committee (approval 322-51) and the Shionogi ethics committee (approval 2020-082-02).

Gene expression profiles of the mouse liver

Gene expression levels in the liver were analysed in accordance with a previous study [18].

Statistical analysis

See supplementary material.

Results

Real-world data analysis

Although pellagra causes photosensitivity and nausea, and PFD is a putative cause of both phenotypes in patients with IPF, whether adverse effects caused by PFD are directly related to pellagra remains unclear. However, the following results support our pellagra hypothesis.

Using real-world data analysis, PFD appeared to cause photosensitivity compared with more than 3500 types of registered medicines. In detail, there were 14 411 registrations for photosensitivity from these medicines without PFD and 619 from PFD. The percentage frequency of photosensitivity in patients without PFD was 0.13% and that in patients with PFD was 3.38% (OR 26.0, 95% CI 25.5–30.0), which was a significant increase (figure 2a). Real-world data enabled us to identify putative drug "A", namely niacin or a certain type of vitamin (figure 2b). When niacin and PFD were used together, the percentage frequency was 0% (0/33). When a multivitamin and PFD were used together, the percentage frequency was 1.74% (8/460). A significant decrease in the number of registrations of this side-effect was not observed in patients cotreated with niacin or a multivitamin with PFD compared with those treated with PFD alone. However, there was a tendency of a decrease in these combination groups. Furthermore, in real-world data analysis, PFD and nintedanib, which are anti-fibrotic agents used for patients with IPF, appeared to cause gastrointestinal events, such as nausea, fatigue, decreased appetite and diarrhoea. In detail, there were 1 806 392 registrations for these gastrointestinal events from medicines without PFD, 11 156 from PFD and 9528 from nintedanib. The percentage frequency of these events in patients without PFD was 8.0% and that in patients with PFD was 42.4% (OR 8.5, 95% CI 8.2–8.7), which was a significant increase (figure 2c). The percentage frequency of these events in patients without nintedanib

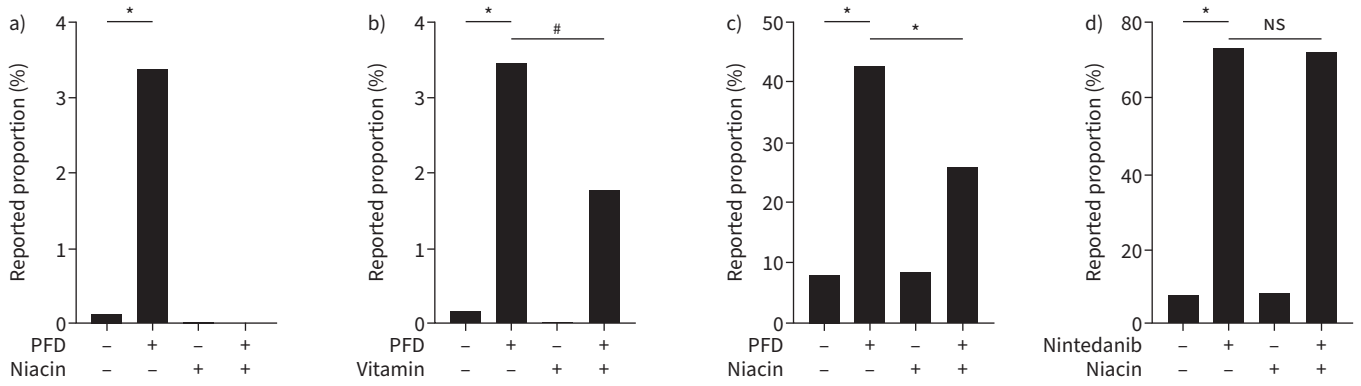

FIGURE 2 Results of the real-world data analysis. The reported proportions of photosensitivity were compared between a) patients who used pirfenidone (PFD) and/or niacin and b) those who used PFD and/or a multivitamin preparation including niacin. Gastrointestinal events, such as nausea, fatigue, decreased appetite and diarrhoea, were compared between c) patients who used PFD and/or niacin and d) those who used nintedanib and/or niacin. *: significant difference ($p<0.05$); #: no significant difference, but there was a tendency; NS: nonsignificant.

was 8.0% and that in patients with nintedanib was 73.2% (OR 31.4, 95% CI 30.0–32.8), which was a significant increase (figure 2d). Real-world data then enabled us to identify putative drug "A", namely niacin (figure 2c). The percentage frequency was 25.9% (15/58) (OR 0.5, 95% CI 0.3–0.9) when niacin and PFD were used together, which was a significant decrease (figure 2c). When niacin and nintedanib were used together, the percentage frequency was 72.0% (18/23) (OR 1.3, 95% CI 0.5–3.6), which was a significant decrease (figure 2d).

Photosensitivity caused by PFD

In accordance with the procedure (supplementary figure S1a), no dermal response, such as oedema (ear swelling) and erythema, to PFD was observed in mice fed a normal or low-niacin diet compared with those without PFD treatment (figure 3a and b). Although the incidence of erythema in patients with IPF using PFD has been reported to be 5% (www.info.pmda.go.jp/go/pack/3999025F1021_1_09), no type of erythema was observed in our experiments. However, some dermal response to ultraviolet (UV) was observed in mice fed a normal or low-niacin diet with PFD (figure 3c and d). As shown in figure 3d, significant ear swelling (oedema) was observed in mice fed the low-niacin diet with PFD 3 mg per mouse compared with those treated with PFD 0 or 1 mg per mouse and those fed the normal diet. Significant erythema was observed in mice fed the low-niacin diet with PFD (1 or 3 mg per mouse) compared with those fed the normal diet and those treated without PFD (figure 3d). Our results indicated that niacin status played a major role in the development of PFD-related photosensitivity, which appeared similar to pellagra. We also analysed skin levels of arachidonic acid metabolites by liquid chromatography-mass spectrometry (LC/MS)-based methods to evaluate this dermal response. This analysis was performed because certain types of oxidised fatty acids may be major players in the development of pellagra-related photosensitivity. A higher PGE2 response to UV irradiation was not observed in mice fed the low-niacin diet with PFD (3 mg per mouse) compared with those without irradiation (figure 3e and supplementary table S1), even though PGE2 has been recognised to play a major role in the development of this type of photosensitivity. However, a higher 18-hydroxyeicosatetraenoic acid (HETE), 11,12-DHET and 14,15-DHET response to UV was observed in mice fed the low-niacin diet with PFD 3 mg per mouse than in those treated with PFD 0 or 1 mg per mouse or those fed the normal diet (figure 3e and supplementary table S1). Interestingly, high DHET levels are thought to be an indicator of inflammation [23].

Nausea caused by PFD

Body weight in mice was significantly increased from day 3 compared with day 1 (figure 4a) and, unexpectedly, there was no difference in body weight loss (or gain) among the groups (figure 4b). However, body weight in mice fed the low-niacin diet with PFD (3 mg per mouse) at day 13 was significantly lower than that in those fed the normal diet with PFD (3 mg per mouse) and those treated with vehicle (figure 4a). In accordance with the procedure of the mouse experiment (supplementary figure S1b) and previous studies [6, 18, 24–26], we analysed pica behaviour using faeces to assess nausea quantitatively. Pica was observed in mice fed the low-niacin diet with PFD (3 mg per mouse) as faecal whitening from day 15 (figure 4c). Paper strips stained with carminic acid were then used to collect coloured faeces from mice with nausea at days 5 and 6 onwards after the first PFD administration to also

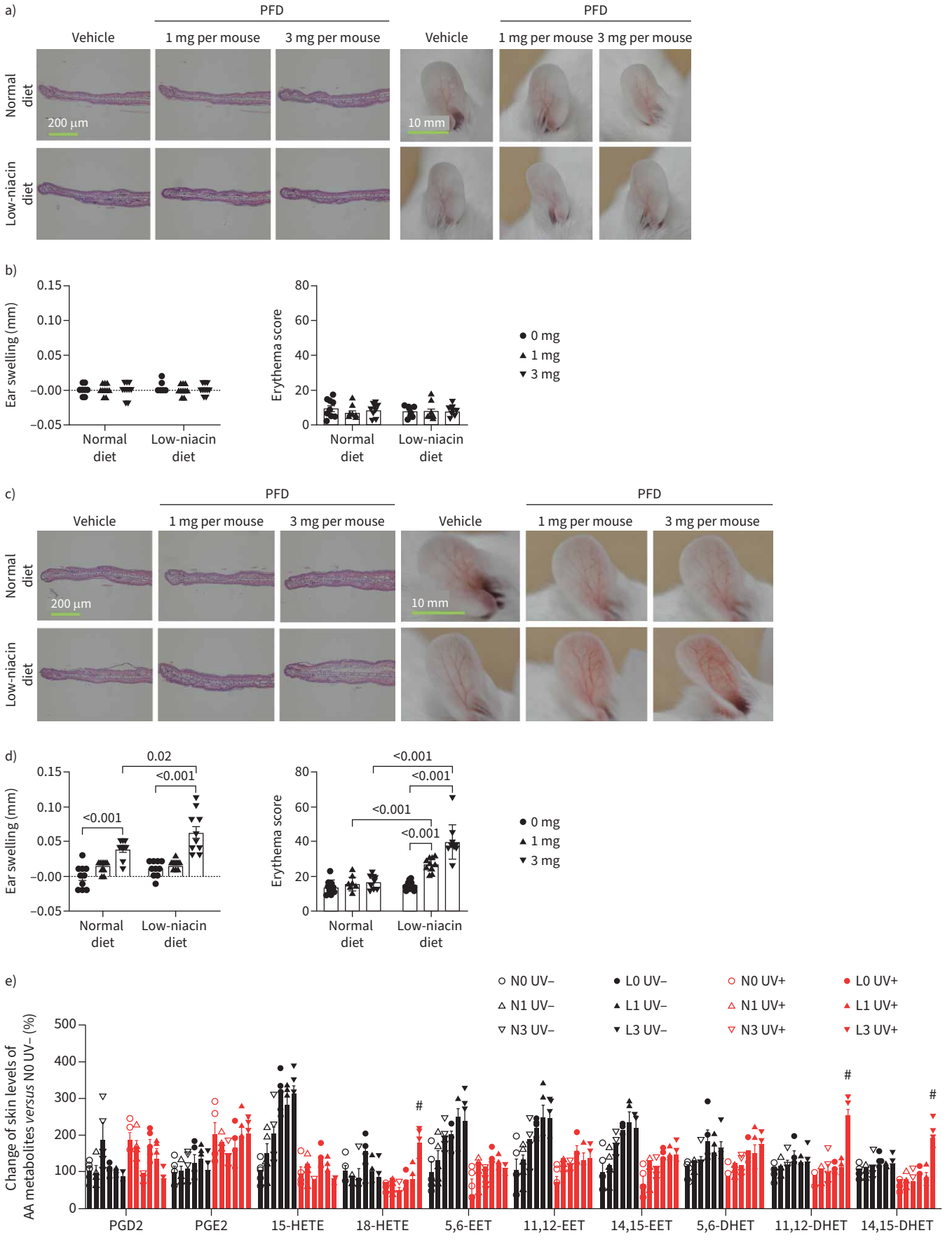
a)
PFD
PFD
Vehicle
1 mg per mouse
3 mg per mouse
Vehicle
1 mg per mouse
3 mg per mouse
Normal diet
Low-niacin diet
200 µm
10 mm
b)
Ear swelling (mm)
0.15
0.10
0.05
−0.00
−0.05
Normal diet
Low-niacin diet
Erythema score
80
60
40
20
0
0 mg
1 mg
3 mg
c)
PFD
PFD
Vehicle
1 mg per mouse
3 mg per mouse
Vehicle
1 mg per mouse
3 mg per mouse
Normal diet
Low-niacin diet
200 µm
10 mm
d)
Ear swelling (mm)
0.02
<0.001
<0.001
Normal diet
Low-niacin diet
Erythema score
<0.001
<0.001
<0.001
<0.001
0 mg
1 mg
3 mg
e)
N0 UV−
N1 UV−
N3 UV−
L0 UV−
L1 UV−
L3 UV−
N0 UV+
N1 UV+
N3 UV+
L0 UV+
L1 UV+
L3 UV+
Change of skin levels of AA metabolites versus N0 UV− (%)
500
400
300
200
100
0
#
#
#
PGD2
PGE2
15-HETE
18-HETE
5,6-EET
11,12-EET
14,15-EET
5,6-DHET
11,12-DHET
14,15-DHET

FIGURE 3 Pellagra-related photosensitivity caused by pirfenidone (PFD). Histological and clinical features of mice treated with or without PFD at day 16 are shown. a, c) Histological and clinical features of mice a) without or c) with ultraviolet (UV) irradiation at day 16. b) No dermal response, such as oedema (ear swelling) and erythema, was observed in mice fed the normal diet with PFD. d) Some dermal responses, such as oedema (ear swelling) and erythema, to UV irradiation were observed in mice fed the normal diet with PFD or the low-niacin diet with PFD. p-values are indicated. e) Skin levels of arachidonic acid (AA) metabolites were analysed by liquid chromatography-mass spectrometry-based methods in mice fed the normal diet (N) or the low-niacin diet (L) with 0 (vehicle), 1 or 3 mg PFD per mouse without UV irradiation (UV–) or with UV irradiation (UV+). Vertical bars indicate the percentage change in each metabolite *versus* the average of mice fed the normal diet with vehicle without UV irradiation (N0 UV–). The results of the statistical analysis are shown in supplementary table S1. Multiple comparisons were carried out by two-way ANOVA (Tukey–Kramer *post hoc* test). #: we focused on 18-hydroxyeicosatetraenoic acid (18-HETE), 11,12-dihydroxyeicosatrienoic acid (11,12-DHET) and 14,15-DHET. PG: prostaglandin; EET: epoxyeicosatrienoic acid. Data represent mean±SE (n=5). Statistical analysis was carried out as described in the supplementary material. The experiments were repeated twice.

assess nausea quantitatively. Faeces from each mouse (n=4) were analysed and prominent behaviour was only seen in mice fed the low-niacin diet with PFD (figure 4d). The nausea score was significantly higher in mice fed the low-niacin diet with PFD 3 mg per mouse than in those treated with vehicle or PFD 1 mg per mouse and with those fed the normal diet at days 15 and 16 (figure 4e). Furthermore, this score was significantly higher in mice fed the low-niacin diet with PFD 1 mg per mouse than in those treated with vehicle or fed the normal diet at day 16.

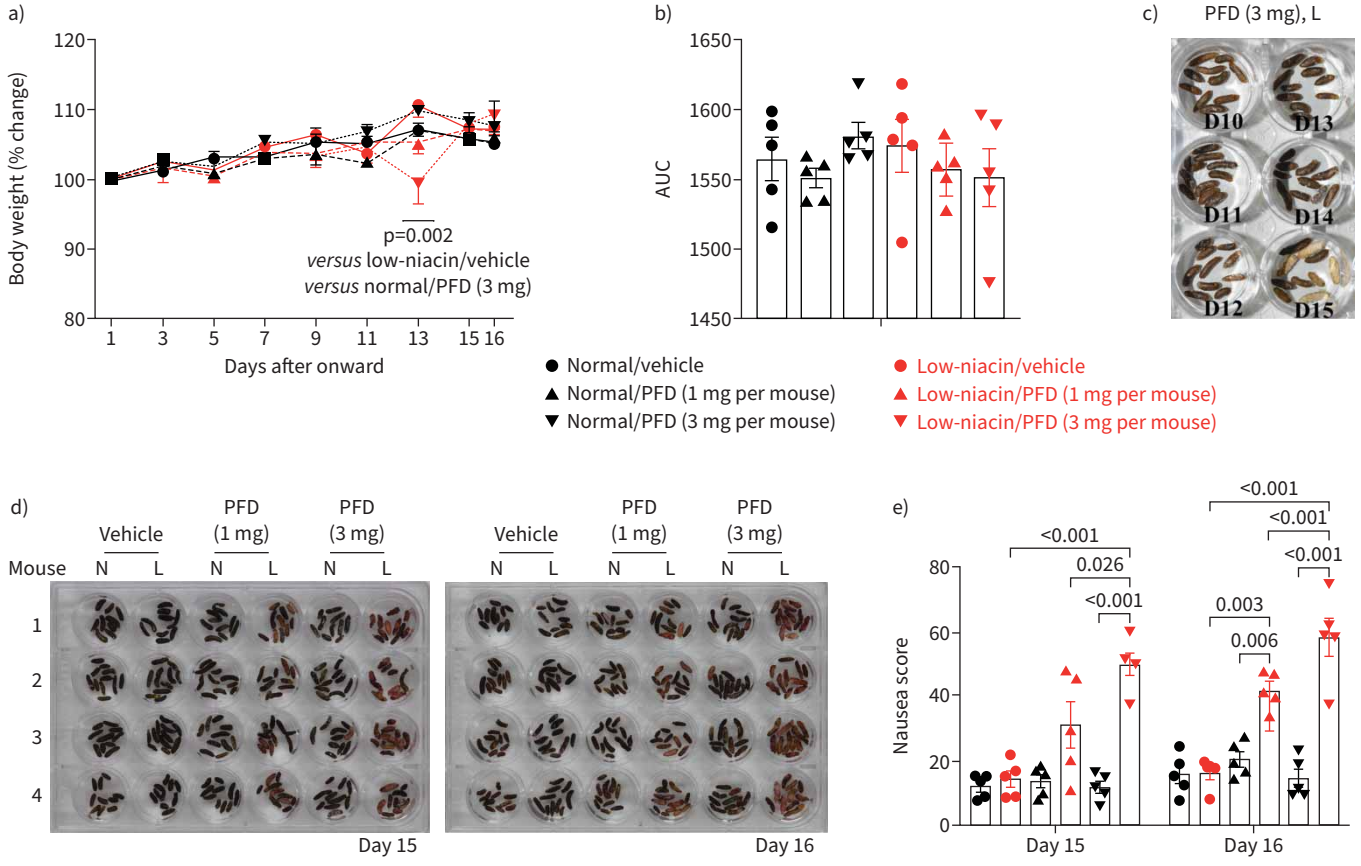

FIGURE 4 Pellagra-related nausea caused by pirfenidone (PFD) (3 mg per mouse). a) Growth curves (body weight % change) in mice fed the normal diet with PFD or the low-niacin diet with PFD (n=5 in each group). b) Each comparison was carried out between both groups. AUC: area under the curve. c) The effect of pica on the shape of faeces from day 10 to 15 (D10–D15) was evaluated in mice treated with PFD kept under the low-niacin diet. Some faeces are indicated and pica began at 5 days onward after the first administration. d) Mice were kept under paper strips stained with carminic acid and coloured faeces were used to evaluate the severity of nausea. Some faeces from mice (n=4) at days 5 and 6 onwards are shown, and nausea severity (nausea score) was evaluated quantitatively using the coloured faeces (n=5). e) Multiple comparisons were carried out by two-way ANOVA (Tukey–Kramer *post hoc* test). Data are presented as mean±SE (n=5). Statistical analysis was carried out as described in the supplementary material. The experiments were repeated twice.

Gene expression profiles

In accordance with the procedure of the mouse experiment (supplementary figure S1B), harvested liver samples from each group, *i.e.* normal diet with vehicle, normal diet with PFD (3 mg per mouse), low-niacin diet with vehicle and low-niacin diet with PFD (3 mg per mouse) groups, were used to validate pica behaviour. To evaluate genes that fluctuate before the appearance of pica, liver samples were harvested from mice at day 13 just before the beginning of pica. A cluster dendrogram shows the hierarchical relationships among the mouse groups (figure 5a). We found that the normal diet with vehicle or PFD groups were the most similar. Similar to the low-niacin diet with vehicle and the normal diet with vehicle or PFD groups, we also found that the low-niacin diet with PFD group was far from the other groups. These results suggested that a certain type of synergic effect of both treatments was observed. A volcano plot was used to identify changes in large datasets between the mouse groups and plot significance *versus* fold change on the *y*- and *x*-axes, respectively (figure 5b). All genes were sorted in accordance with the significance of the p-values and the top 50 genes are shown in supplementary

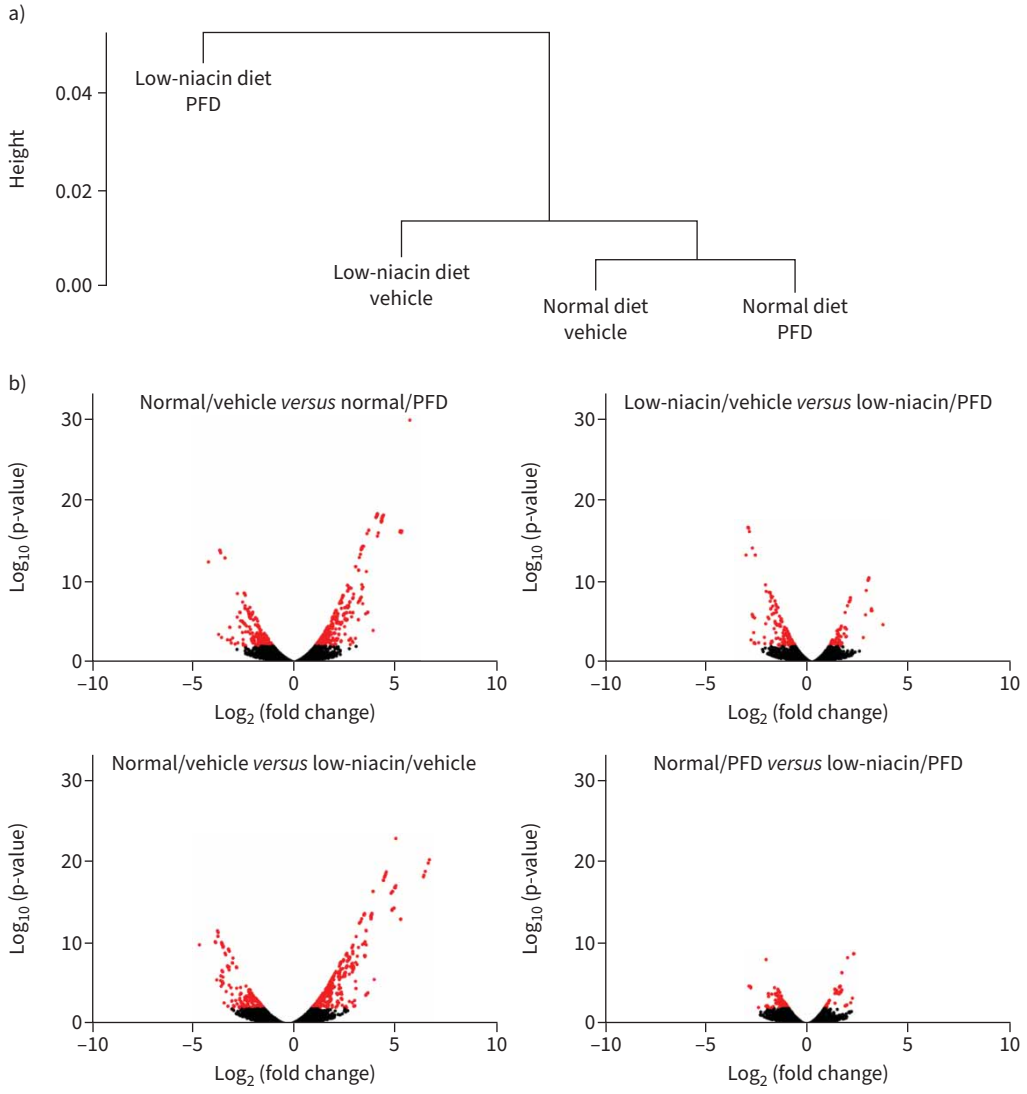

FIGURE 5 Mouse liver gene expression profiles. a) A cluster dendrogram from gene analysis was prepared to confirm genetic similarity among mice fed the low-niacin diet with vehicle or pirfenidone (PFD) (3 mg per mouse) and mice fed the normal diet with vehicle or PFD. b) Volcano plots between mice fed the normal diet with vehicle and those treated with PFD, mice fed the low-niacin diet with vehicle and those treated with PFD, mice fed the normal diet with vehicle and those fed the low-niacin diet, and mice fed the normal diet with PFD and those fed the low-niacin diet. Each dot represents one gene. Red dots indicate genes with significantly increased or decreased expression between the comparisons.

tables S2–S5 (supplementary table S2 was presented in our previous study [18]). All listed genes are shown in table 2. When the gene profile of the liver in mice fed the normal diet with vehicle was compared with that of mice treated with PFD or fed the low-niacin diet, nicotinamide *N*-methyltransferase (NNMT) was extracted. A high NNMT response to the low-niacin diet or PFD was observed. When the profile in mice fed the normal diet with PFD was compared with that in mice fed the low-niacin diet or in those fed the low-niacin diet with vehicle or PFD, higher liver levels of nausea-related gene expression were extracted [27].

Urine levels of metabolites as a biomarker of pellagra

Urine levels of niacin metabolites are thought to be diagnostic markers for pellagra [28]. Therefore, the tryptophan–nicotinamide pathway and related metabolites were analysed (supplementary figure S2). We performed targeted metabolomics in accordance with previous studies [6, 18] in urine from mice to investigate whether PFD-related photosensitivity is similar to pellagra. Urine levels of nicotinamide (NAM) in mice fed the normal diet with PFD 3 mg per mouse were significantly higher than those in mice fed the normal diet treated with vehicle or PFD 1 mg per mouse at days 15 and 16 (figure 6a and b). Furthermore, urine levels of NAM in mice fed the low-niacin diet with PFD 3 mg per mouse were significantly higher than those in mice fed the low-niacin diet treated with vehicle or PFD 1 mg per mouse at day 16. Urine levels of *S*-adenosylmethionine (SAM) in patients with IPF treated with PFD tended to be higher than those in patients with IPF treated without PFD (p=0.07) (figure 6c), although the variation was larger compared with that seen in mice. Interestingly, both metabolites (NAM and SAM) are substrates of NNMT. These results suggest that PFD promotes pellagra-like symptoms *via* niacin deficiency.

TABLE 2 Main data from comprehensive genetic analysis

Comparison	Gene	Log_2 (fold change)	p-value	Ranking[#]
Normal/vehicle *versus* normal/PFD	*Nnmt*	2.67	0.00000	27
Normal/vehicle *versus* low-niacin/vehicle	*Nnmt*	1.87	0.00005	32
Normal/PFD *versus* low-niacin/PFD	*Neat1*	1.99	0.00000	4
	Cyp7a1	−1.99	0.00000	5
	Socs2	1.63	0.00002	7
	Idi1	−1.58	0.00003	8
	Sult2a1	1.41	0.00008	12
	Slc25a30	1.44	0.00012	15
	Hgf	−1.28	0.00093	27
	Erbb4	−1.51	0.00212	37
	Fdps	−1.01	0.00427	49
Low-niacin/vehicle *versus* low-niacin/PFD	*Alas1*	3.82	0.00000	3
	Lpin1	3.40	0.00000	4
	Mt2	3.26	0.00000	6
	Dbp	3.47	0.00000	8
	Chka	−3.48	0.00000	13
	Efna1	−3.40	0.00000	15
	Por	2.52	0.00000	17
	Socs2	2.68	0.00000	19
	Per1	2.64	0.00000	21
	Fkbp5	2.36	0.00000	23
	Fmo3	2.21	0.00000	26
	Pfkfb3	2.55	0.00000	27
	Arntl	−3.24	0.00000	29
	Cebpb	2.25	0.00000	32
	Thrsp	2.01	0.00000	35
	Wee1	2.43	0.00000	39
	St3gal5	1.90	0.00000	40
	Gadd45g	2.26	0.00000	42
	Fmo5	1.85	0.00000	43
	Angptl4	1.91	0.00000	45
	Stbd1	1.72	0.00001	50

[#]: each gene was sorted by the p-value and the top 50 genes were ranked (supplementary tables S2–S5); the main genes of interest are listed here, ranked within each comparison. PFD: pirfenidone.

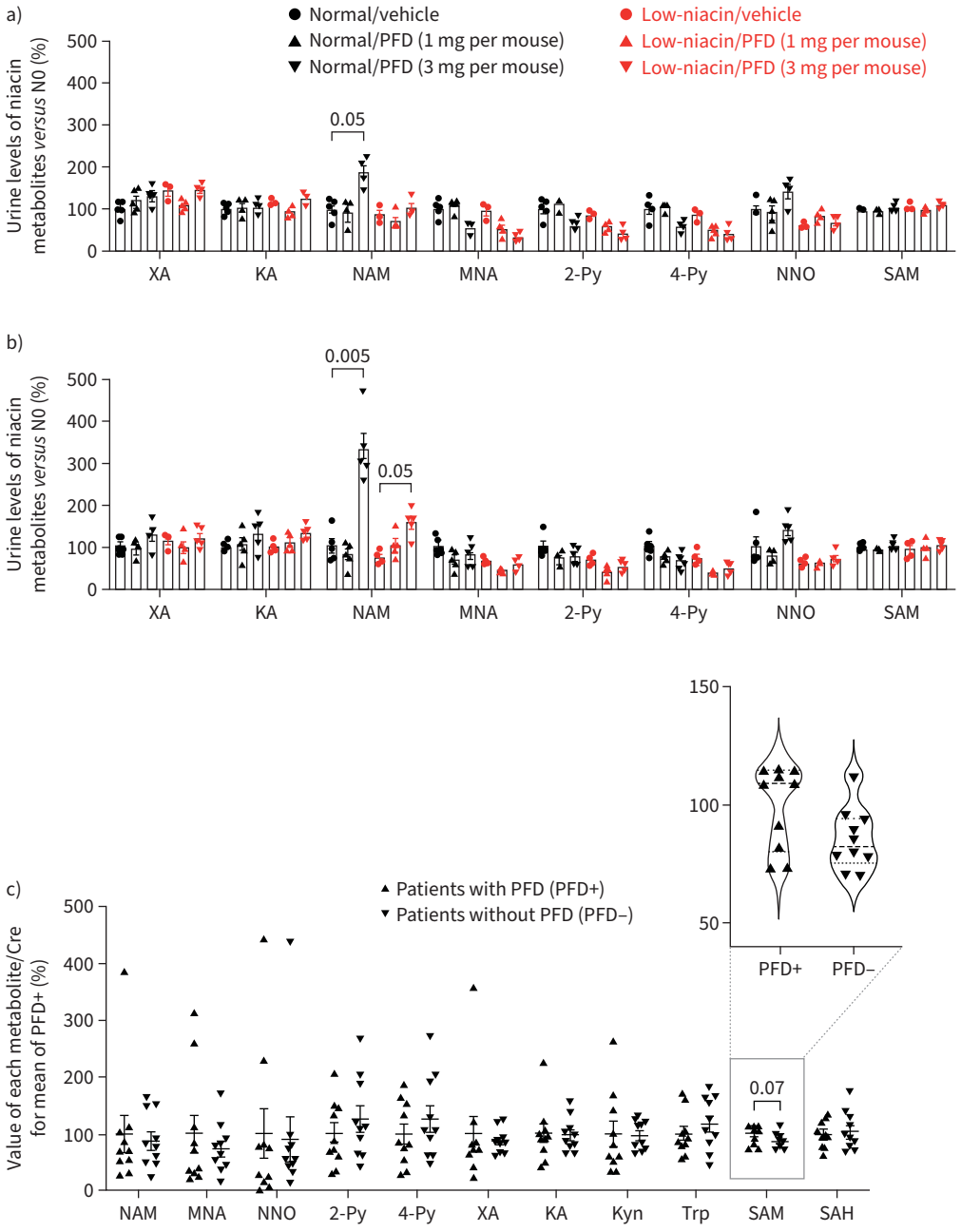

FIGURE 6 Urine levels of niacin and its metabolites. The value (%) of each metabolite, based on metabolite/creatinine (Cre) ratios, was measured in mice fed the normal diet with pirfenidone (PFD) or low-niacin diet with PFD: a) day 15 and b) day 16. N0: normal diet with vehicle. c) The value (%) of each metabolite was measured in idiopathic pulmonary fibrosis patients (n=10 in each group). XA: xanthurenic acid; KA: kynurenic acid; NAM: nicotinamide; MNA: *N*-methylnicotinamide; 2-Py: *N*′-methyl-2-pyridone-3-carboxamide; 4-Py: *N*′-methyl-4-pyridone-3-carboxamide; NNO: nicotinamide-*N*-oxide; SAM: *S*-adenosylmethionine; Kyn: kynurenic acid; Trp: tryptophan; SAH: *S*-adenosylhomocysteine. Comparisons were carried out by one- or two-way ANOVA (Mann–Whitney test or Tukey–Kramer *post hoc* test). Data are presented as mean±SE. Statistical analysis was carried out as described in the supplementary material. The mouse experiments were repeated twice.

Discussion

Although pellagra causes photosensitivity and nausea [6–9], and PFD is a putative cause of these symptoms [6–9], whether certain types of PFD-related symptoms are directly related to pellagra is unclear. In this study, the results of real-world data analysis support our hypothesis that PFD causes pellagra-like phenotypes. Importantly, the results from the pharmacological studies also indicated that photosensitivity

and nausea caused by PFD were similar to pellagra. Furthermore, high responses of NNMT substrates, such as NAM or SAM, to PFD were observed in urine from humans and mice. Although the types of these substrates were different between the species, we believe that PDF inhibited this enzymatic activity. This possibility is supported by our finding that higher levels of gene expression were seen in mice treated with PFD than in those treated with vehicle. These results indicate the homology and similarity of PFD-related symptoms with a certain type of pellagra that was caused by compromising the pathway involved in niacin metabolism [9].

Pellagra is caused by malnutrition and has two types. One type may result from an insufficient intake of dietary niacin or tryptophan (primary pellagra) and the other results from insufficient use of niacin or tryptophan (secondary pellagra) [9]. Interestingly, patients with IPF are thought to be malnourished [29], which might be partially true because almost all patients with IPF are older. Indeed, urine levels of niacin metabolites, such as *N*-methylnicotinamide and *S*-adenosylhomocysteine, which are metabolites of NNMT, were significantly different between older subjects and younger subjects (supplementary figure S3). However, urine levels of NNMT substrates, such as NAM and SAM, were not different between the groups (supplementary figure S3). Furthermore, these metabolites were not changed in older mice compared with younger mice (supplementary figure S4). In accordance with these results, a low-niacin diet was used to mimic the niacin status of older people. Another reason for using a low-niacin diet is that a niacin-deficient diet causes body weight loss in rats and severe pathological conditions such as pellagra [30, 31]. To prevent these severe phenotypes in our model, we used a low-niacin diet, but not a niacin-free diet. We found that the low-niacin diet did not cause body weight loss or pellagra in mice. This finding suggested that the low levels of niacin provided by this modified diet were sufficient to prevent the appearance of pellagra symptoms in this mouse model.

Using the established mouse model of pre-pellagra [18], we found that PFD caused pellagra-related photosensitivity and we analysed skin arachidonic acid metabolite levels by LC/MS-based methods to evaluate this dermal response. A higher PGE2 response to UV irradiation was not observed in mice fed the low-niacin diet with PFD compared with those without irradiation. This finding is inconsistent with previous findings that PGE2 plays a major role in the development of this type of photosensitivity [18, 22]. This discrepancy between studies might be partially due to the fact that the dermal response to UV irradiation in a previous animal model was more obvious [18] compared with that in our model or due to a difference in mouse strains. We did not use the black strain used in these previous studies, in accordance with another previous study [17], because quantitative observation of erythema is too difficult. We used white mice, such as BALB/c, to investigate erythema. A higher 18-HETE, 11,12-DHET and 14,15-DHET response to UV was observed in mice fed the low-niacin diet with PFD than in those treated with vehicle or fed the normal diet. Interestingly, higher DHET levels are thought to be a biomarker of inflammation [23]. PFD has an antagonistic effect on transient receptor potential cation channel subfamily V member 4 (TRPV4) [32] and EET is thought to be an endogenous ligand of TRPV4 [33]. In contrast to our expectations, no significant quantitative changes in EETs were observed in mice treated with PFD compared with those treated without PFD. PGE2 may be important in the development of pellagra-related dermatitis [17]. A high PGE2 response to pellagra-related symptoms was not observed in our mice model (figure 3e). Modulation of niacin metabolism induces the modulation of fatty acid metabolism independent of pellagra [18]. These results suggest that high PGE2 metabolism is not a cause of PFD-induced adverse effects, but is caused by a defect in niacin metabolism.

Using an *in vivo* murine nausea model [6, 18], we found that PFD caused pellagra-related nausea. When we compared INH and PFD as causes of pellagra-related nausea, some phenotypes induced by both compounds appeared to be different from each other [6, 11]. A higher SAM or NAM response was observed in urine from mice treated with PFD than in those treated with vehicle, but a response to INH was not observed [6, 11, 18]. However, there were significantly lower kynurenic acid and xanthurenic acid responses in urine from mice treated with INH than in those treated with vehicle, but a response to PFD was not observed. At least under our experimental conditions, there was an earlier onset of pica behaviour after the beginning of the experiments in the INH group than in the PFD group. Furthermore, pellagra caused by PFD was milder than that caused by INH. We could not continue pharmacological experiments to cause pellagra using INH for $\geqslant$6 days because mice would have died. However, death of mice was not observed in the experiments using PFD [6, 11, 18]. Although there is currently no detailed molecular mechanism that can explain these differences between PFD and INH, the points of competition in niacin metabolism differ between these two drugs. INH responds to vitamin B6, while PFD appears to respond to more downstream metabolites. Whether the differences between ordinal and PFD-related pellagra are qualitative or quantitative is unknown. However, we defined PFD-induced pellagra as mild [11, 18], which is thought to be a cause of the lack of dementia in PFD-induced pellagra. Despite these differences, no

pellagra-related behaviour was identified in either group treated with PFD and INH under proper niacin-containing feeding conditions, and we believe that both groups met the conditions of pellagra [6–9].

The onset mechanism of the side-effects has not been fully determined and many patients with IPF have reduced doses or even cessation of PFD because of these side-effects [34]. When considering the quality of life (QoL) of patients, clarifying the mechanism and preventing side-effects are important. The analysis of real-world data in this study showed that PFD and nintedanib had common side-effects, but their responsiveness to niacin was significantly different. Studies have shown that patients with IPF are poorly nourished [29]. Therefore, we conclude that nutritional improvement, including niacin status, may be important to maintain the QoL of these patients. Accordingly, we believe that new putative methodologies need to be developed to prevent such effects and maintain the QoL of patients. We have discussed the utility of niacin from the viewpoint of maintaining the patient's QoL using PFD. However, whether niacin inhibits the efficacy of PFD needs to be determined. Animal experiments have shown that niacin suppresses bleomycin-induced lung fibrosis [35]. Interestingly, niacin has an inhibitory effect on TRPV4 [36] and plays a major role in the development of pulmonary fibrosis [34]. Furthermore, an inhibitory effect of PFD on TRPV4 may contribute to its therapeutic effects [32], and if a combination therapy of PFD and niacin can be developed, a synergistic therapeutic effect may be expected.

These results suggest that PFD causes pellagra-like symptoms, such as INH [4, 6], because niacin status appears to be critical for the development of nausea and photosensitivity. Patients with IPF are thought to suffer from malnutrition [29], but individual data on niacin status had not been obtained until this study. The details of IPF from a nutritional viewpoint need to be determined. Another aspect that needs to be determined is whether drug "A" unexpectedly lowers the incidence of drug-induced adverse effects. Drug "A" could be a practical remedy for adverse effects in humans, which would enable repositioning of an approved drug "A" to lower the pharmacological risk. Finally, further studies are required to investigate whether niacin prevents adverse effects in patients with IPF who receive PFD and its mechanisms should be clarified.

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