The breast cancer resistance protein protects against a major chlorophyll-derived dietary phototoxin and protoporphyria

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The breast cancer resistance protein (BCRP/ABCG2) is a member of the ATP-binding cassette family of drug transporters and confers resistance to various anticancer drugs. We show here that mice lacking Bcrp1/Abcg2 become extremely sensitive to the dietary chlorophyll-breakdown product pheophorbide a, resulting in severe, sometimes lethal phototoxic lesions on light-exposed skin. Pheophorbide a occurs in various plant-derived foods and food supplements. Bcrp1 transports pheophorbide a and is highly efficient in limiting its uptake from ingested food. *Bcrp1*$^{-/-}$ mice also displayed a previously unknown type of protoporphyria. Erythrocyte levels of the heme precursor and phototoxin protoporphyrin IX, which is structurally related to pheophorbide a, were increased 10-fold. Transplantation with wild-type bone marrow cured the protoporphyria and reduced the phototoxin sensitivity of *Bcrp1*$^{-/-}$ mice. These results indicate that humans or animals with low or absent BCRP activity may be at increased risk for developing protoporphyria and diet-dependent phototoxicity and provide a striking illustration of the importance of drug transporters in protection from toxicity of normal food constituents.

Members of the ATP-binding cassette (ABC) family of drug transporters actively export many drugs and toxins from cells. Their presence at strategic sites in the body such as the intestine, blood–brain barrier, and placenta protects the organism by limiting the systemic penetration and tissue toxicity of xenotoxins (1–3). The breast cancer resistance protein (BCRP/ABCG2) and its mouse homologue Bcrp1 transport various anticancer drugs including topotecan, mitoxantrone and doxorubicin, thus causing multidrug resistance in cancer cells (3). We previously found that application of BCRP inhibitors to mice enhanced the oral uptake and fetal penetration of topotecan, suggesting that Bcrp1 provides an efficient pharmacologic barrier at these sites (4). Coadministration of BCRP inhibitors has also been tested in patients for its ability to improve anticancer chemotherapy by enhancing oral uptake and possibly tumor penetration of BCRP substrate drugs. The first results are promising, but they also revealed unanticipated toxicity (5). It thus is important to establish the risks associated with chronic inhibition of BCRP.

To study the physiological and pharmacological functions of BCRP, we generated *Bcrp1* knockout mice. Absence of Bcrp1 resulted in a striking sensitivity to the dietary chlorophyll catabolite pheophorbide a, which made these mice extremely photosensitive. Moreover, *Bcrp1* knockout mice displayed a previously uncharacterized type of protoporphyria, a group of metabolic disorders frequently associated with skin photosensitivity in patients (6). Pheophorbide a and protoporphyrin are structurally related and belong to the porphyrins, a broad class of molecules that include the "pigments of life": chlorophyll, heme, and cobalamin (6). Our data show that BCRP is physiologically important in processes involving handling of porphyrins, and we expect that a partial or complete deficiency for BCRP may contribute to several porphyrin-related phototoxicities in humans and animals.

Materials and Methods

Animals. Mice were housed and handled according to institutional guidelines complying with Dutch legislation. Animals used in this study were *Bcrp1*$^{-/-}$ and wild-type mice of a comparable genetic background (FVB or mixed 129/Ola and FVB) between 9 and 14 weeks of age. Animals were kept in a temperature-controlled environment with a 12-h light/12-h dark cycle. They received a standard (AM-II) or semisynthetic (reference 4068.02) diet (Hope Farms, Woerden, The Netherlands) and acidified water ad libitum.

Materials. Topotecan and [^{14}C]topotecan [56 Ci/mol (1 Ci = 37 GBq)] were from GlaxoSmithKline (King of Prussia, PA). Pheophorbide a was from Frontier Scientific/Porphyrin Products (Logan, UT).

Generation of Bcrp1$^{-/-}$ Mice. By using *Bcrp1* cDNA probes, a 129/Ola mouse genomic sequence containing exons 1–8 of *Bcrp1* was identified. A 5.1-kb fragment containing exons 3–6, encoding most of the ATP-binding domain, was deleted and replaced with a 1.8-kb *pgk-hygro* cassette in reverse-transcriptional orientation. Electroporation and selection for recombinant E14 embryonic stem cells was done as described (7). Of 161 hygromycin-resistant clones, 18 were targeted correctly as confirmed by Southern analysis of *Sca*I-digested genomic DNA with 3′ and 5′ *Bcrp1* probes (Fig. 1*a*). The absence of additional *pgk-hygro* cassettes inserted elsewhere in the genome was confirmed by hybridization with a *hygro*-specific probe. Chimeric mice were generated by microinjection of two independently targeted embryonic stem cell clones into blastocysts. Chimeric offspring were backcrossed to FVB mice. By using this approach, two independent *Bcrp1*$^{-/-}$ mouse lines were established.

Clinical Chemical Analysis of Plasma. Standard clinical chemistry analyses on plasma were performed on a Hitachi 911 analyzer to determine levels of bilirubin, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, creatinine, urea, Na^+, K^+, Ca^{2+}, Cl^-, phosphate, total protein, and albumin.

Abbreviations: ABC, ATP-binding cassette; BCRP/ABCG2, breast cancer resistance protein; Bcrp1/Abcg2, murine BCRP; PPIX, protoporphyrin IX.

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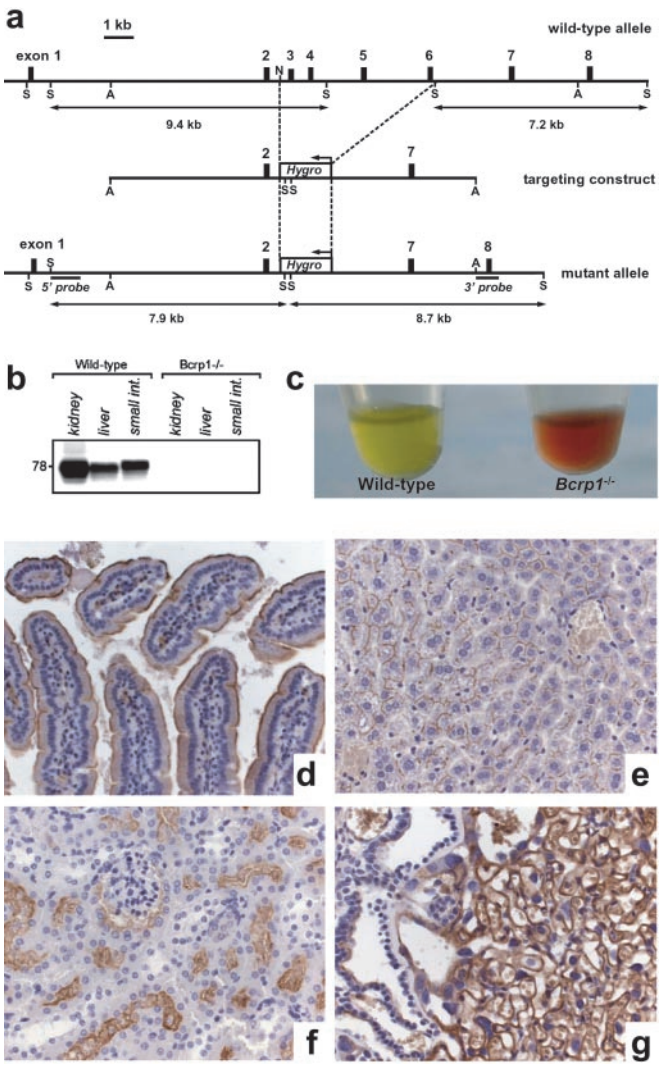

Fig. 1. Generation and analysis of *Bcrp1*$^{-/-}$ mice. (*a*) A 5.1-kb fragment containing exons 3–6 (exons are indicated by filled boxes) was replaced with an inverted *pgk-hygro* cassette. Restriction sites: S, *Sca*I; A, *Asp*718; N, *Nhe*I. For Southern analysis, 5′ and 3′ probes were used on *Sca*I-digested genomic DNA. Diagnostic restriction fragments are indicated by double-headed arrows. (*b*) Western analysis on crude membrane fractions from liver, kidney, and small intestine (20 μg per lane). (*c*) Bile from wild-type and *Bcrp1*$^{-/-}$ mice. (*d–g*) Immunohistochemical detection (×40) of Bcrp1 in small intestine (*d*), liver (*e*), kidney (*f*), and placenta (*g*).

Hematological Analysis. Hemoglobin, mean corpuscular volume, red and white blood cells, and platelets were determined in peripheral blood on a Cell Dyn 1200 analyzer (Abbott).

HPLC Analysis. Levels of topotecan, pheophorbide a, and protoporphyrin IX (PPIX) were determined by HPLC analysis as described (4, 8, 9). The method described for the determination of pheophorbide a was modified slightly. A Luna C18(2) column (250 × 4.6-mm i.d., 5-μm particle size, Phenomenex, Torrance, CA) protected with a guard column (10 × 3-mm i.d.) packed with reversed-phase material (Varian Chrompack) was used for the separation.

Generation of mAbs. A fusion gene consisting of the gene for the *Escherichia coli* maltose-binding protein and a fragment encoding amino acids 221–394 of the mouse *Bcrp1* gene was constructed in the pMAL-c vector. Production and purification of the fusion protein, immunization of rats, and fusion protocols were as described (10, 11). Results are shown for mAb BXP-9 or BXP-53, which worked well on immunoblots and in immunohistochemistry.

Western Analysis. Crude membrane fractions from tissues were prepared as described (12). Western blotting was performed as described (7). Blots were probed with mAb BXP-9 (1:10). mAb binding was detected by using peroxidase-conjugated rabbit anti-rat IgG (1:1,000, DAKO).

Histological Analysis and Immunohistochemistry. Tissues were fixed in 4% phosphate-buffered formalin, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin according to standard procedures. For immunohistochemistry, tissues were deparaffinized in xylene and rehydrated. Endogenous peroxidase activity was blocked by using 3% (vol/vol) H_2O_2 in methanol for 10 min. Before staining, paraffin sections were pretreated by heat-induced epitope retrieval. Slides were incubated with 5% normal goat serum/PBS for 30 min, and subsequently sections were incubated overnight with a 1:400 dilution of BXP-53 at 4°C. mAb immunoreactivity was detected with the streptavidin-biotin immunoperoxidase (sABC) method by using biotinylated goat anti-rat IgG (Dako, 1:100) as secondary antibody, and diaminobenzidine substrate for visualization. After counterstaining with hematoxylin, slides were mounted. For negative control, the primary mAb was omitted.

***In Vitro* Pheophorbide a Accumulation Assay.** Exponentially growing cells were incubated for 1 h at 37°C in normal medium in the presence of 10 μM pheophorbide a with or without 10 μM Ko143. Cells were trypsinized, washed, and suspended in Hanks' solution with 1% FCS. Light exposure was minimized, and after trypsinization all procedures were done at 4°C. Relative cellular accumulation of pheophorbide a was determined by flow cytometry using a FACScan (Becton Dickinson) with excitation at 488 nm and emission detection at 650 nm.

Pharmacokinetic Experiments. Pharmacokinetic experiments were performed as described (4, 7). For fetal accumulation studies, *Bcrp1*$^{+/-}$/*Mdr1a/b*$^{-/-}$ mice were obtained by appropriate backcrossing of *Bcrp1*$^{-/-}$ mice to mice deficient for the drug transporting *P*-glycoproteins Mdr1a and Mdr1b (*Mdr1a/b*$^{-/-}$ mice). Subsequently, *Bcrp1*$^{+/-}$/*Mdr1a/b*$^{-/-}$ mice were crossed to generate *Bcrp1*$^{+/+}$, *Bcrp1*$^{+/-}$, and *Bcrp1*$^{-/-}$ fetuses in a *Mdr1a/b*$^{-/-}$ background.

Photosensitivity Experiments. Mice were exposed to standard fluorescent light (Philips TLD50W/84HF) with a 12-h light/12-h dark cycle. The distance from the light source was 130 cm, resulting in an exposure of ≈1,000 lux. These light conditions are comparable to those in an artificially well lit indoor office space.

Bone Marrow Transplantation. Bone marrow was harvested from femurs and suspended in Hanks' solution with 1% FCS. Bone marrow cells (3×10^6) were transplanted via the lateral tail vein to recipient mice that had received 9 Gy of whole-body irradiation. After transplantation mice were kept for 8 weeks in filter-top cages. The genotype of peripheral blood was confirmed by PCR analysis.

Results

Generation and Analysis of *Bcrp1*$^{-/-}$ Mice. To study the *in vivo* roles of Bcrp1, we generated constitutive *Bcrp1* knockout mice (Fig. 1*a*). Northern and Western blots confirmed the absence of Bcrp1 mRNA (not shown) and protein in *Bcrp1*$^{-/-}$ mice (Fig. 1*b*). Immunohistochemically, Bcrp1 was detected in apical membranes of epithelial cells of small intestinal villi and renal

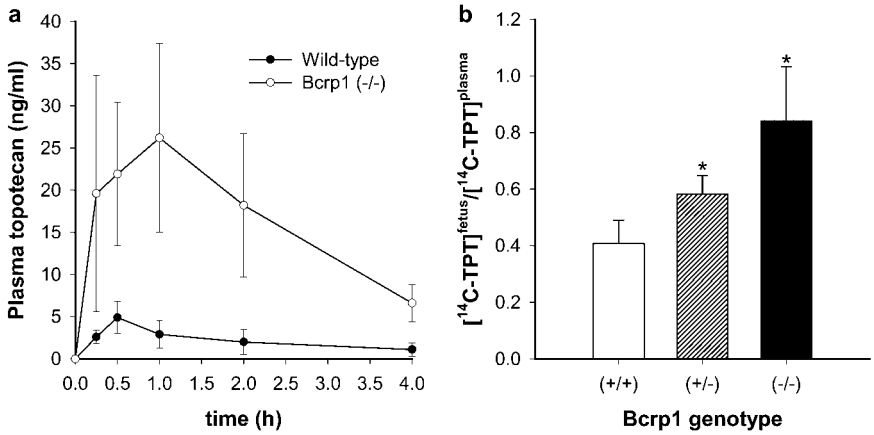

Fig. 2. Pharmacologic effects of Bcrp1. (*a*) Plasma concentration versus time curve after oral administration of 1 mg/kg topotecan to mice (means ± SD, n = 5–6; $P < 0.001$ for area under the curves, Student's t test). (*b*) Ratio of [^{14}C]topotecan concentration in fetus over maternal plasma at 15 min after i.v. administration of 0.2 mg/kg [^{14}C]topotecan to pregnant dams (gestation day 15.5; mean ± SD, n = 3 dams and 11 *Bcrp1*$^{-/-}$, 13 *Bcrp1*$^{+/-}$, and 7 *Bcrp1*$^{+/+}$ fetuses; *, $P < 0.001$ compared with *Bcrp1*$^{+/+}$ fetuses, Student's t test).

proximal tubules, hepatic bile canalicular membranes, and placental labyrinth cells of wild-type (Fig. 1 *d–g*) but not *Bcrp1*$^{-/-}$ (not shown) mice.

Bcrp1$^{-/-}$ mice were fertile, their life spans and body weights were not different from wild-type, they were born at the expected Mendelian ratio, and they did not demonstrate phenotypic aberrations under standard housing conditions. Unlike some other members of the ABCG subfamily (13), Bcrp1 seemed not to be critically involved in lipid metabolism, because we did not observe changes in plasma levels of cholesterol and phospholipids. Hematological and plasma clinical chemical analysis revealed no abnormalities except that unconjugated bilirubin was increased in *Bcrp1*$^{-/-}$ mice (1.50 ± 0.55 μM in wild type versus 6.50 ± 1.05 μM in *Bcrp1*$^{-/-}$). Surprisingly, the bile of *Bcrp1*$^{-/-}$ mice was red instead of yellow (Fig. 1*c*). Both the increased levels of unconjugated bilirubin and red-colored bile were diet-dependent, because they disappeared in mice that received a semisynthetic diet consisting of purified nutrients. However, HPLC analysis indicated that the red bile color was not caused by bilirubin or its conjugates. As will be described below, it is likely to be caused by a chlorophyll catabolite.

Role of Bcrp1 in Oral Availability and Fetal Accumulation of Topotecan. We studied the pharmacologic role of Bcrp1 by determination of the oral availability and fetal accumulation of topotecan (Fig. 2). The oral availability of topotecan was increased ≈6-fold in *Bcrp1*$^{-/-}$ mice, indicating that intestinal Bcrp1 limits the uptake of topotecan. Fetal accumulation of topotecan was measured in fetuses from a cross between *Bcrp1*$^{+/-}$ and *Mdr1a/b*$^{-/-}$ mice to avoid confounding effects of Mdr1a/b *P*-glycoprotein (4). Fig. 2*b* shows that the ratio of fetal topotecan concentration to maternal plasma concentration was ≈2-fold higher for *Bcrp1*$^{-/-}$ fetuses compared with *Bcrp1*$^{+/+}$ fetuses, whereas *Bcrp1*$^{+/-}$ fetuses showed an intermediate accumulation. These results show that Bcrp1 in the placenta can limit the penetration of substrate drugs from the maternal plasma into the fetus.

Diet-Dependent Phototoxicity in *Bcrp1*$^{-/-}$ Mice. The *Bcrp1*$^{-/-}$ mice had not displayed any visible phenotypical aberration until a few *Bcrp1*$^{-/-}$ mice suddenly developed severe necrotic ear lesions (Fig. 3 *a–e*). Only mice housed on the top shelf, closest to the light source, developed these lesions, suggesting some form of phototoxicity. Further analysis showed that all *Bcrp1*$^{-/-}$ mice developed ear lesions when exposed to standard fluorescent light but only when fed with one particular batch of food. Phototoxic ear lesions developed 1 week after feeding with this "phototoxic" batch, and in some cases lesions also appeared on the tail, snout, and rims of the eyes. Phototoxicity was never observed in wild-type mice.

Diet-dependent photosensitization is common and can be caused by a variety of chemicals including drugs and pesticides but also by natural toxins derived from plants and fungi. The primary plant component present in our standard mouse diet was alfalfa (*Medicago sativa*) leaf concentrate. Outbreaks of photosensitization in cattle are reported frequently and are often associated with the ingestion of alfalfa. This phototoxicity has been attributed to biochemical conversion products or mycotoxins generated in humidly stored alfalfa (14). To investigate whether alfalfa was the source of the phototoxicity in *Bcrp1*$^{-/-}$ mice, we fed them diets containing increased amounts of alfalfa. Whereas no phototoxicity was observed at normal (5%) levels of alfalfa, all *Bcrp1*$^{-/-}$ (but not wild-type) mice progressively developed phototoxic lesions at higher (10% and 20%) alfalfa dosages (Fig. 3*f*). Thus, twice the normal amount of this batch of alfalfa already induced phototoxicity in *Bcrp1*$^{-/-}$ mice.

Bcrp1$^{-/-}$ Mice Are Extremely Sensitive to the Phototoxin Pheophorbide a. It has been shown that high levels of pheophorbide a, a phototoxic porphyrin catabolite of chlorophyll, can be formed by alfalfa chlorophyllase depending on the treatment of the alfalfa during storage and processing (refs. 15 and 16; Fig. 3*g*). To test whether *Bcrp1*$^{-/-}$ mice were sensitive to pheophorbide a, we determined its phototoxicity after oral administration (Table 1). Whereas phototoxicity (or other toxicity) was never observed in wild-type mice up to 200 mg/kg/day, the *Bcrp1*$^{-/-}$ mice displayed a dramatic hypersensitivity. The lowest dose at which phototoxicity occurred in *Bcrp1*$^{-/-}$ mice was 2 mg/kg/day, indicating that *Bcrp1*$^{-/-}$ mice are at least 100-fold more sensitive to pheophorbide a. At 16 mg/kg/day, ear lesions developed already after 2 days, and after 3 days mice developed severe edema of the head and became moribund. The hypersensitivity of the *Bcrp1*$^{-/-}$ mice to pheophorbide a corresponded with highly increased plasma levels. Plasma levels of pheophorbide a were 17-fold (10.40 ± 2.74 μg/ml) and 24-fold (14.54 ± 2.50 μg/ml) higher, respectively, in *Bcrp1*$^{-/-}$ mice fed with phototoxic or 20% alfalfa food compared with a "normal" food batch (0.61 ± 0.47 μg/ml). In wild-type mice, plasma levels of pheophorbide a were undetectable on any of these diets.

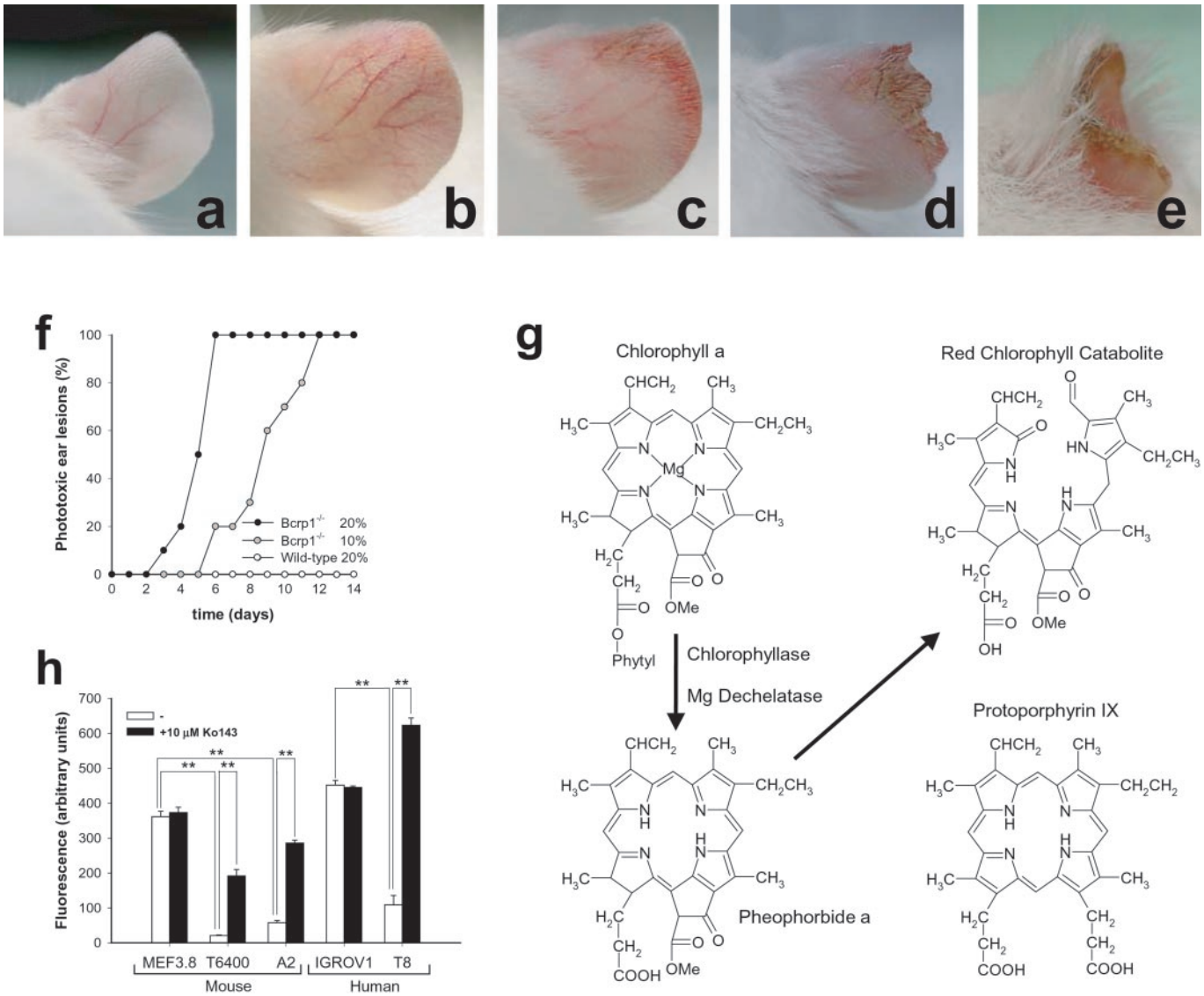

Fig. 3. Phototoxicity and transport of pheophorbide a. (*a*) Normal ear. (*b–e*) Progression of phototoxic ear lesions in a period of 3–5 days in *Bcrp1*$^{-/-}$ mice. (*f*) Incidence of phototoxic ear lesions on diet containing 10% and 20% alfalfa ($n = 10$ mice). (*g*) Chlorophyll breakdown in plants, reactions, and enzymes are indicated with an arrow. Also shown is the structure of PPIX. (*h*) Pheophorbide a accumulation in cells exposed to pheophorbide a (10 μM) for 1 h at 37°C in the presence or absence of Bcrp1/BCRP-inhibitor Ko143 (10 μM) (mean fluorescence ± SD, $n = 3$; **, $P < 0.01$, Student's *t* test).

Pheophorbide a Is Transported by Bcrp1/BCRP. To determine whether pheophorbide a is transported by Bcrp1/BCRP, we measured its accumulation in cell lines by flow cytometry (Fig. 3*h*). We used the mouse fibroblast line MEF3.8 and its derivatives T6400 and A2 that overexpress *Bcrp1* by drug selection or transduction with *Bcrp1* cDNA, respectively (17, 18). Accumulation of pheophorbide a was reduced 18-fold in T6400 cells compared with MEF3.8. This effect could be largely reversed by the specific Bcrp1/BCRP inhibitor Ko143 (19). We obtained similar results for the A2 cell line and the human IGROV1 ovarian cancer cell line and its *BCRP*-overexpressing derivative T8 (20), indicating that pheophorbide a is transported efficiently by both murine Bcrp1 and human BCRP. Thus, Bcrp1 efficiently reduces the bioavailability of dietary pheophorbide a by preventing its uptake from the intestine and possibly by mediating its elimination via liver and kidney. Without this efficient barrier, (natural) fluctuations of pheophorbide a in the diet can lead to phototoxicity. The phototoxic food batch must have contained relatively high pheophorbide a levels, most likely resulting from variations in treatment of the alfalfa ingredient.

Interestingly, in plants, degradation of pheophorbide a by cleavage of the porphyrin ring yields a red chlorophyll catabolite (ref. 21; Fig. 3*g*). In *Bcrp1*$^{-/-}$ mice that received a semisynthetic diet and thus had yellow bile, the red bile color reappeared after oral administration of (dark-green) pheophorbide a, suggesting that the red compound excreted in the bile of *Bcrp1*$^{-/-}$ mice (Fig. 1*c*) is red chlorophyll catabolite or a related pheophorbide a metabolite.

No Abnormalities in Differentiation of Bone Marrow Cells in *Bcrp1*$^{-/-}$ Mice. It has been shown that *Bcrp1* is also expressed in hematopoietic stem cells and erythroid precursor cells in the bone marrow and that it is responsible for the "side-population" (SP) phenotype, associated with primitive stem cells and caused by active extrusion of the fluorescent dye Hoechst 33342 (22). We also detected Bcrp1 in mature murine erythrocytes (not shown). Although the physiologic function of Bcrp1 in hematopoietic (stem) cells is still unknown, Zhou *et al.* (22) speculated that it might be involved in hematopoietic differentiation. However, by

Table 1. Pheophorbide a phototoxicity in mice

Dosage, mg/kg/day	Wild-type	*Bcrp1*$^{-/-}$
1	na	—
2	na	$+^{11}$
4	na	$+^{8}$
8	—	$+^{5}$
16	—	$+^{2*}$
32	—	na
64	—	na
100	—	na
200	—	na

Pheophorbide a was administered orally for up to 12 days to mice fed with "normal" food (n = 3 per group). na, Not analyzed; —, no phototoxicity observed; +, phototoxicity observed; superscript numbers, average number of days until phototoxicity was first observed.

*Moribund mice were killed after 3 days. Light conditions are specified in *Materials and Methods.*

flow cytometry we found no abnormalities in the relative numbers of erythroid precursors (Ter119$^+$), granulocytes (Gr1$^+$), macrophages (Mac1$^+$), or B cells (B220$^+$) in bone marrow of *Bcrp1*$^{-/-}$ mice. In addition, no significant hematologic differences were observed in peripheral blood (not shown).

Bcrp1$^{-/-}$ Mice Display a Previously Unknown Type of Protoporphyria. In humans, well known causes of phototoxicity are genetic defects in the heme biosynthetic pathway that result in the accumulation of photosensitizing porphyrins in the skin. These porphyrins are structurally highly related to pheophorbide a. We therefore determined levels of various endogenous porphyrins, including PPIX (Fig. 3*g*), the immediate precursor of heme, in erythrocytes, plasma, liver, bile, and urine. We found that erythrocyte levels of PPIX were increased 10-fold in *Bcrp1*$^{-/-}$ mice. This increase was seen in mice kept on all diets (Fig. 4*a*) including the semisynthetic diet, with which the mice do not display obvious photosensitivity. This result implies that the increased level of PPIX was independent of the diet, and that this level in itself was not sufficient to cause marked photosensitivity. Plasma PPIX was also increased, but no significant differences in levels of PPIX in liver or bile were observed between the groups (not shown). Bcrp1 expressed in erythrocytes and their precursors apparently protects these cells from excessive accumulation of PPIX, possibly by extrusion of this compound.

Protoporphyria Can Be Cured by Bone Marrow Transplantation. We next transplanted lethally irradiated wild-type and *Bcrp1*$^{-/-}$ mice with bone marrow from either genotype and determined the effects on protoporphyria and photosensitivity. Eight weeks after transplantation, erythrocyte levels of PPIX in *Bcrp1*$^{-/-}$ mice with wild-type transplants were comparable with those of wild-type mice, whereas wild-type mice with *Bcrp1*$^{-/-}$ transplants were comparable with *Bcrp1*$^{-/-}$ mice (Fig. 4*b*). The *Bcrp1*$^{-/-}$ protoporphyria is thus a bone marrow-autonomous phenotype that can be cured by transplantation with wild-type bone marrow, and that does not depend on Bcrp1 activity elsewhere in the body. Wild-type mice transplanted with *Bcrp1*$^{-/-}$ (or wild-type) bone marrow were not photosensitive when fed a 20% alfalfa diet, whereas *Bcrp1*$^{-/-}$ recipients of either bone marrow genotype did display photosensitivity (Fig. 4*c*). However, *Bcrp1*$^{-/-}$ mice with *Bcrp1*$^{-/-}$ transplants (with protoporphyria) were more photosensitive than *Bcrp1*$^{-/-}$ mice with wild-type transplants (no protoporphyria; Fig. 4*c*), suggesting that PPIX may have contributed to the photosensitivity in *Bcrp1*$^{-/-}$ mice.

Discussion

Our data provide a striking illustration of the importance of the ABC transporter Bcrp1 in protection from a normal food constituent such as pheophorbide a and at the same time emphasize the unpredictability of exposure to such constituents. Given the amount of chlorophyll ingested by most omnivores and herbivores, it is not surprising that they are normally well protected from its toxic breakdown products. In humans, pheophorbide a-induced phototoxicity has been reported after ingestion of chlorella tablets (23), a dried algae preparation taken as "natural health supplement" by millions of people. Several pickled vegetables also contain substantial amounts of pheophorbide a and have been shown to cause phototoxicity in rats (23). In the human population there are many incompletely understood incidences of idiosyncratic food and drug hypersensitivities including phototoxicity (24–26). In light of our data it will be of great interest to investigate whether part of these hypersensitivities could be explained by partial or complete deficiencies in the activity of BCRP, *P*-glycoprotein, or related ABC transporters.

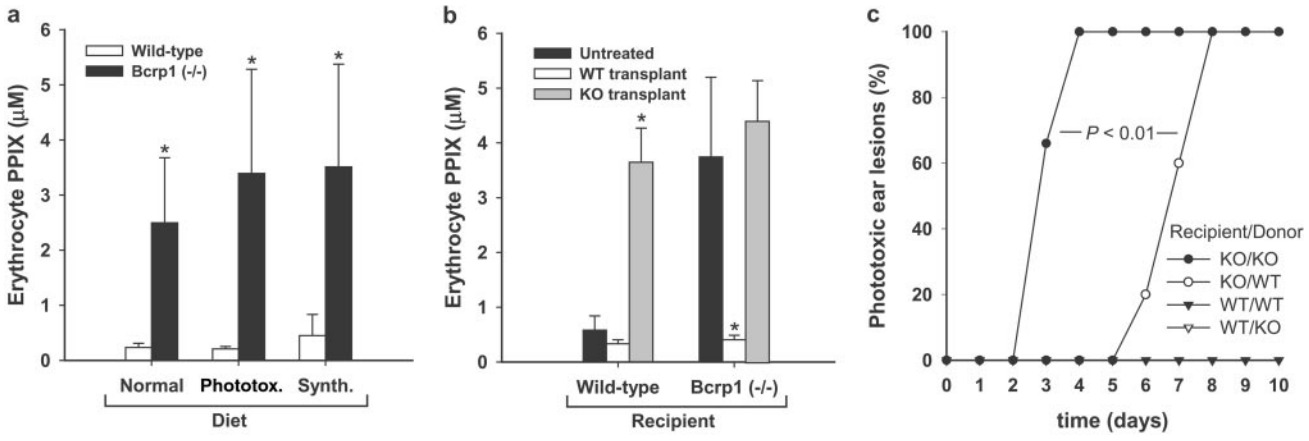

Fig. 4. Effect of bone marrow transplantation on protoporphyria and phototoxicity. (*a*) Erythrocyte levels of PPIX in *Bcrp1*$^{-/-}$ and wild-type mice receiving normal, phototoxic (Phototox.), or semisynthetic (Synth.) diet (n = 5). (*b*) Erythrocyte levels of PPIX after bone marrow transplantation (*, $P < 0.05$, Student's t test). KO, knockout. (*c*) Incidence of phototoxic ear lesions in wild-type and *Bcrp1*$^{-/-}$ mice (recipients) transplanted with *Bcrp1*$^{-/-}$ or wild-type (donors) bone marrow. To induce phototoxicity, mice were fed a 20% alfalfa diet (n = 3–5; $P < 0.01$ for difference between knockout/knockout and knockout/wild type, Student's t test).

Our results demonstrate unequivocally that Bcrp1 affects the pharmacologic behavior of the anticancer drug topotecan, potentially limiting the therapeutic efficacy of it and related clinically used drugs, for instance by restricting their oral availability. Our current and previously obtained data indicate that this problem can be circumvented by strategic application of effective BCRP inhibitors (4, 5). However, this study also indicates that prolonged inhibition of BCRP activity might reveal unexpected sensitivities to compounds that are commonly present in the diet. Ongoing clinical trials with BCRP inhibitors should therefore be monitored carefully for such unexpected toxicity events.

Our study further shows that BCRP is important for the handling of endogenous porphyrins. The *Bcrp1*$^{-/-}$ mice display a previously unknown type of genetic porphyria characterized by increased levels of PPIX in erythrocytes. This porphyria is unique because it is not caused by a defect in one of the enzymes of the heme biosynthetic pathway, in contrast to all previously identified genetic porphyrias (6). Erythropoietic protoporphyria for instance also leads to accumulation of PPIX in erythrocytes but is caused by a deficiency of ferrochelatase, the enzyme that mediates the conversion of PPIX into heme. Severe erythropoietic protoporphyria results in marked photosensitivity. We do not understand the exact mechanism behind the protoporphyria caused by Bcrp1 deficiency yet, but the structural similarity with pheophorbide a suggests that excess PPIX (or perhaps another porphyrin) may be cleared from erythroid cells by BCRP. Low or absent BCRP activity may exacerbate the consequences of other genetic or drug-induced protoporphyrias and thus could explain some of the highly variable penetrance seen in these syndromes (6).

After completion of this study, Zhou *et al.* (27) published an analysis of an independently generated *Bcrp1* knockout mouse. In line with our findings, no abnormalities were observed in the hematopoiesis of these mice. No mention was made of other physiological abnormalities except that hematopoietic cells were unable to extrude the dye Hoechst 33342 and displayed an increased sensitivity to the drug mitoxantrone (27). We note that the phenotypes we observed in our *Bcrp1*$^{-/-}$ mice may well have been missed unless specifically looked for or were only apparent under specific (dietary and light) conditions.

In conclusion, we have shown that BCRP is involved in physiologically important processes involving the handling of exogenous and endogenous porphyrins. Reduced BCRP activity as a consequence of mutation, inhibition, or down-regulation thus might contribute to diet-induced phototoxicity, protoporphyria, and possibly other porphyrin-related toxicities and disorders.

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