



HAL
open science

Stiripentol protects against calcium oxalate nephrolithiasis and ethylene glycol poisoning

Marine Le Dudal, Léa Huguet, Joëlle Perez, Sophie Vandermeersch, Elise Boudierlique, Ellie Tang, Carole Martori, Nicole Chemaly, Rima Nabbout, Jean-Philippe Haymann, et al.

► To cite this version:

Marine Le Dudal, Léa Huguet, Joëlle Perez, Sophie Vandermeersch, Elise Boudierlique, et al.. Stiripentol protects against calcium oxalate nephrolithiasis and ethylene glycol poisoning. *Journal of Clinical Investigation*, 2019, 129 (6), pp.2571-2577. 10.1172/JCI99822 . hal-03180756

HAL Id: hal-03180756

<https://hal.sorbonne-universite.fr/hal-03180756v1>

Submitted on 25 Mar 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Stiripentol protects against calcium oxalate nephrolithiasis and ethylene glycol poisoning

Marine Le Dudal,^{1,2,3} Léa Huguet,^{1,2} Joëlle Perez,^{1,2} Sophie Vandermeersch,^{1,2} Elise Boudierlique,^{1,2} Ellie Tang,^{1,2} Carole Martori,⁴ Nicole Chemaly,⁵ Rima Nabbout,⁵ Jean-Philippe Haymann,^{1,2,4} Vincent Frochot,⁴ Laurent Baud,^{1,2,4} Georges Deschênes,⁶ Michel Daudon^{1,2,4}, Emmanuel Letavernier,^{1,2,4}

¹ Sorbonne Universités, UPMC Univ Paris 06, UMR S 1155, F-75020, Paris, France

² INSERM, UMR S 1155, F-75020, Paris, France

³ Department of Pathology, Ecole Nationale Vétérinaire d'Alfort, Université Paris-Est, 94704 Maisons-Alfort, France

⁴ Physiology Unit, AP-HP, Hôpital Tenon, F-75020, Paris, France

⁵ Pediatric Neurology Unit, Hôpital Necker, AP-HP and Paris Descartes University, F-75015, Paris, France

⁶ Pediatric Nephrology Unit, Hôpital Robert Debré, AP-HP and Paris Diderot University, F-75019, Paris, France

AUTHOR INFORMATION

Correspondence:

Emmanuel LETAVERNIER, Service des Explorations Fonctionnelles Multidisciplinaires, Hôpital TENON, 4 rue de la Chine, 75020 Paris (France). Phone 33 1 56 01 67 73 ; Fax 33 1 56 01 70 03. E-mail: emmanuel.letavernier@aphp.fr

Running Title: Stiripentol decreases urinary oxalate

Word Count: 2664

Number of Figures and Tables: Figures: 3 ; Table: 1

Keywords: Stiripentol, Oxalate, Kidney, Nephrolithiasis, Ethylene glycol, Primary hyperoxaluria

Conflict of Interest statement:

A patent relative to the use of Stiripentol as a treatment against nephrolithiasis and ethylene glycol poisoning has been deposited by EL and MD (WO2017140658 A1).

No other conflict of interest

Abstract

Increased urinary oxalate excretion (hyperoxaluria) promotes the formation of calcium oxalate crystals. Monogenic diseases due to hepatic enzymes deficiency result in chronic hyperoxaluria, promoting end-stage renal disease in children and young adults. Ethylene glycol poisoning also results in hyperoxaluria promoting acute renal failure and frequently death. Stiripentol is an antiepileptic drug used to treat children affected by Dravet syndrome, possibly by inhibiting neuronal lactate dehydrogenase-5 isoenzyme. As this isoenzyme is also the last step of hepatic oxalate production, we hypothesized that Stiripentol would potentially reduce hepatic oxalate production and urine oxalate excretion.

In vitro, Stiripentol decreased in a dose-dependent manner the synthesis of oxalate by hepatocytes. *In vivo*, Stiripentol oral administration reduced significantly urine oxalate excretion in rats. Stiripentol protected kidneys against calcium oxalate crystal deposits in acute ethylene glycol intoxication and chronic calcium oxalate nephropathy models. In both models, Stiripentol improved significantly renal function. Patients affected by Dravet syndrome and treated with Stiripentol have a lower urine oxalate excretion than control patients. A young girl affected by severe type I hyperoxaluria received Stiripentol for several weeks: urine oxalate excretion decreased by two-thirds. Stiripentol is a promising potential therapy against genetic hyperoxaluria and ethylene glycol poisoning.

Introduction

Urine oxalate excretion is low (normal value <0.5 mmol/day in human) but its affinity for calcium ions make it a major promoter of calcium oxalate crystals and kidney stones formation.¹ Calcium oxalate precipitation in kidney tubules is particularly fateful when resulting from acute intoxication by ethylene glycol or from genetic disorders.^{2,3} Actually, primary hyperoxaluria is a rare but severe genetic enzymatic defect increasing glyoxylate and oxalate hepatic production; oxalate precipitates in kidney tubules, leading to end-stage kidney disease and poor prognosis in young patients.

Oxalate is produced in the liver from glyoxylate transformation by lactate dehydrogenase (LDH) type 5 isoenzyme and excreted in urine, with little biological control.⁴ To date, there is no drug reducing oxalate production by liver. In 2015, Sada et al. demonstrated that Stiripentol, an antiepileptic drug, targets lactate production by LDH-5 isoenzymes in neurons in vitro.⁵ We hypothesized that this drug could also inhibit hepatic LDH-5 isoenzymes and oxalate production by liver, and thereby decrease urinary oxalate excretion. Stiripentol is a safe drug that has been used for years in addition to other antiepileptic drugs to target seizures in Dravet syndrome, a rare form of epilepsy affecting young children.⁶

Results and Discussion

To investigate whether Stiripentol could decrease hepatic oxalate production, hepatocytes (HepG2 cells) were grown *in vitro* in hydroxyproline-enriched cell culture medium (Figure 1A).⁷ The exposure of hepatocytes to Stiripentol concentrations at “therapeutic” levels (5 to 100 µg/mL) resulted in a dose dependent and significant decrease in oxalate production (* p= 0.03, n=4 experiments, Figure 1A). As a matter of comparison, recommended dose of Stiripentol in Dravet syndrome is usually 1000-3000 mg/day in children, giving serum levels of 4-22 µg/mL.⁸ As the drug has high first pass metabolism in liver, it may even be hypothesized that relatively low doses of Stiripentol could inhibit hepatic LDH activity efficiently without significant inhibition of systemic LDH-5 isoenzymes. To confirm the implication of LDH-5 isoenzyme, we performed SiRNA experiments targeting LDH-A mRNA (sub-unit of LDH-5). SiRNA suppressed 60 to 70 % of LDH-A mRNA and decreased significantly oxalate production. The addition of 10 µg/mL Stiripentol to SiRNA decreased mildly oxalate production by HepG2 cells, suggesting that most of oxalate synthesis was actually due to LDH-5 isoenzyme activity (Figure 1B).

We next analysed whether Stiripentol would also be efficient to reduce urine oxalate excretion *in vivo*. Six rats received orally 200 mg/Kg/day Stiripentol during 48 hours. Urine oxalate/creatinine excretion decreased slightly but significantly from 0.16 ± 0.01 mmol/mmol before treatment to 0.11 ± 0.01 mmol/mmol (* p=0.002). After a 4 days wash-out period, urine oxalate excretion was similar to baseline values (Figure 1C).

Ethylene glycol is present in anti-freeze and is a frequent cause of poisoning, causing more than 5000 intoxications in USA each year.² Its toxicity depends mainly of its transformation into oxalic acid by liver, which precipitates as calcium oxalate in kidneys promoting acute

renal failure and acidosis. Ethylene glycol is first metabolized to glycolaldehyde by the enzyme alcohol dehydrogenase, and then undergoes oxidation to glycolate, glyoxylate, and at last oxalate. To date, the specific treatment of ethylene glycol poisoning is fomepizole or 4-methylpyrazole, a competitive inhibitor of alcohol dehydrogenase in the liver.⁹ The use of Stiripentol as a therapy against ethylene glycol intoxication would be of interest since acting downstream of the metabolic pathway, and could therefore be still efficient after relatively long period following intoxication, even when high amounts of glycolaldehyde have already been produced. Six rats were exposed to ethylene glycol alone and six rats received both ethylene glycol and Stiripentol at 300 mg/kg at the same time (and Stiripentol in drinking water during the next 2 days). Animals were sacrificed 2 days later. Animals exposed to ethylene glycol alone were oliguric, but a small amount of urine has been collected at the time of the sacrifice to perform urine crystals analysis. Crystalluria revealed a large number of calcium oxalate monohydrate (COM) crystals in both groups and to a lesser extent calcium oxalate dihydrate crystals (COD) but the mean crystalline volume was 20 times less in animals receiving Stiripentol ($9467 \pm 2259 \mu\text{m}^3/\text{mm}^3$ versus $201766 \pm 95023 \mu\text{m}^3/\text{mm}^3$, $p=0.004$, Figure 2A-B). The kidneys of rats exposed to ethylene glycol alone were pale and voluminous with a mean weight at 1.31 ± 0.11 grams whereas kidneys from animals receiving Stiripentol in addition had a usual red/brown aspect and normal weight at 0.85 ± 0.02 grams (Figure 2C, $*p=0.004$). Stiripentol protected rats against ethylene-glycol-induced renal failure (mean serum creatinine $78.2 \pm 9.6 \mu\text{mol/L}$ versus $297.1 \pm 74.4 \mu\text{mol/L}$, $p=0.002$, Figure 2G).

The other biological features are depicted in table I, evidencing that animals receiving Stiripentol had a trend toward lower metabolic acidosis (less decreased bicarbonate serum levels), and a significantly lower serum anion gap, the consequence of lower circulating oxalate levels in this context of ethylene glycol poisoning. The morphometric analysis of

kidney tissues confirmed that Stiripentol decreased significantly calcium oxalate crystals deposition in kidneys (Figure 2D-F, $p=0.002$). These deposits were stained by Yasue coloration (revealing calcium oxalate crystals) and the analysis of kidney tissue by Fourier transform infrared spectroscopy (FTIR) confirmed that crystalline deposits in renal tubules were made of calcium oxalate monohydrate exclusively (Figure 2H-I).

Blocking oxalate synthesis could be particularly of interest in primary hyperoxaluria, a genetic enzymatic defect resulting from a mutation of *AGXT*, *GRHPR* or *HOGAI* genes, increasing glyoxylate hepatic production which is finally transformed into oxalate by LDH-5.³ Oxalate precipitates in renal tubules, leading to end-stage kidney disease in children and young adults with high morbidity and mortality, often requiring a combined liver-kidney allograft.¹⁰ There is currently no drug decreasing oxalate production by liver with the exception of pyridoxine which may lower urine oxalate excretion in some cases of type I hyperoxaluria.³

Sixteen Sprague Dawley rats received hydroxyproline and calcium in drinking water during 16 days. This classic animal model of hyperoxaluria induces oxalate nephropathy by increasing hepatic oxalate production as the result of mitochondrial hydroxyproline metabolism.¹¹ The oxalate nephropathy is characterized by calcium oxalate intratubular crystal deposits similar to those observed in primary hyperoxaluria.¹¹ Eight of these rats received Stiripentol orally by gavage, once a day during 16 days.

Urine oxalate excretion increased in both groups after exposure to hydroxyproline but urine oxalate was significantly lower in animals receiving Stiripentol in addition to hydroxyproline and calcium (Figure 3A, $p=0.33$ at day 2, $p=0.10$ at day 4, $*p=0.0002$ at day 8, $*p=0.0019$ at day 11, $*p=0.0002$ at day 15). In parallel, urine glycolate excretion increased in both groups but significantly more in rats exposed to Stiripentol (at day 2 and 8), suggesting that Stiripentol actually inhibits the transformation of glyoxylate into oxalate (Supplementary

Figure 1). Crystalluria revealed the presence of calcium oxalate crystals in both groups but the mean crystalline volume was significantly lower in animals treated by Stiripentol (Figure 3B, $p=0.15$ at day 4, $*p=0.015$ at day 8, $p=0.08$ at day 11, $*p=0.007$ at day 15).

Animals were sacrificed after 16 days exposure to hydroxyproline. FTIR analysis revealed the presence of sparse deposits of calcium oxalate monohydrate crystals in renal tubules and kidney morphometric analysis demonstrated that Stiripentol decreased significantly calcium oxalate deposits in kidney tissues (Figure 3C-E $p=0.004$). Renal function was less altered in animals receiving Stiripentol (figure 3F, $p=0.028$).

To assess whether Stiripentol would reduce efficiently oxalate synthesis in humans, urine oxalate excretion was assessed in children affected by Dravet syndrome requiring Stiripentol treatment ($n=8$, median age 6 years) and in children of similar age affected by cystinuria, kidney-stone formers whose stones are not due to oxalate (control group, $n=40$, median age 7.5 years). Urine oxalate excretion was significantly decreased in patients affected by Dravet syndrome, suggesting that Stiripentol lowers urine oxalate excretion, even when urine oxalate concentrations stands within normal range ($p=0.002$, Figure 4A).

A 17-yr old girl affected by severe type I hyperoxaluria (homozygous *AGXT* mutation c.349-350 insG) received Stiripentol to reduce urine oxalate excretion in Robert Debré Hospital (Paris). Renal function was still normal in the absence of acute episode (serum creatinine 65 $\mu\text{mol/L}$, estimated glomerular filtration rate 88 mL/min/1.73m^2) but she was exhausted by recurrent colic nephritis (about once a month), repeated urological procedures and pyelonephritis. Urine oxalate/creatinine ratio was about 0.18-0.20 mmol/mmol . Her brother, affected by the same disease, had a rapid decrease in renal function and was treated with combined liver-kidney transplantation. Considering the evolution of the disease, in accordance with medical team and after parental agreement, Stiripentol was introduced at half-dose (25 mg/kg/day) and urine oxalate excretion decreased rapidly (Figure 4B). A further

decrease in urine oxalate excretion has been observed when Stiripentol therapy was increased at 50 mg/kg/day (urine oxalate/creatinine ratio: 0.068 mmol/mmol), without identified side effect (Figure 4B).

These results bring new hope to treat children affected by primary hyperoxaluria, a disease characterized by high morbidity due to renal failure and systemic deposits of calcium oxalate in the body, and high mortality. The genetic defect may be cured by liver transplant, but this disabling therapy is usually performed as a combined liver-kidney allograft when end-stage renal disease occurs. The main adverse events related to Stiripentol in Dravet patients are nutrition disorders (anorexia, loss of appetite, weight loss), neurological disorders (drowsiness, hyperexcitability) and in some rare cases neutropenia. Most of side-effects disappear when the dose of comedication is decreased (carbamazepine and clobazepam). Actually, Stiripentol interacts with several P450 cytochromes. Fortunately, children affected by primary hyperoxaluria are not supposed to receive other drugs than pyridoxine and potassium citrate.

In summary, Stiripentol, a potent LDH-5 enzyme inhibitor, limits oxalate production by human hepatocytes *in vitro* and in rats *in vivo*, at “therapeutic” levels. Oral administration of Stiripentol protects rats against ethylene glycol poisoning and oxalate nephropathy. Considering the safety profile of this drug and preliminary results in children, it brings a new hope to prevent the renal and systemic consequences of primary hyperoxaluria, a disease associated to a dramatic morbidity and mortality, and to treat ethylene glycol poisoning, deserving clinical studies.

Methods

Cell culture.

HepG2 cells, an hepatoma human cell line (Sigma Aldrich, France), were grown in DMEM medium (Gibco, Invitrogen, Cergy-Pontoise, France) supplemented with 10% fetal bovine serum (Gibco), 1% glutamine, 4.5 g/L glucose and penicillin/streptomycin (Gibco, 5µl/ml). HepG2 cells medium was supplemented with 10 mM hydroxyproline to induce oxalate production as previously described and to various concentrations of Stiripentol (5-100 µg/mL) during 24 hours.⁷ To modulate LDH-A expression, HEPG2 cells were transfected with small interfering RNA (siRNA) targeting LDH-A human gene, 4 siRNAs for Entrez gene 3939: SI02663535 (FlexiTube siRNA Functionally verified, SI00300622 (FlexiTube siRNA Functionally verified), SI04949616 (FlexiTube siRNA), SI04949609 (FlexiTube siRNA) or positive (silencing)-control siRNA Mm/Hs_MAPK1 control siRNA (SI03650318; both from QIAGEN). Briefly, HepG2 cells were seeded in a 100 mm Petri dishes at a density of 4×10^6 cells/ Petri dish in 10 ml DMEM and 10% FBS and directly transfected with 5nM siRNA through the use of 40 µl HiPerFect transfection reagent (QIAGEN). 24h after transfection, cells were stimulated for 24 h, with hydroxyproline (10 mM), with or without Stiripentol (10µg/ml). Cells were then lysed in RLT, and RNA was isolated with an EZ-10 Spin Column Kit (Proteogenix) and reverse transcribed with a First-Strand cDNA Synthesis Kit (Thermo Scientific). cDNA was amplified on a Light Cycler 480 system (Roche) using SYBR Green (Roche) and specific primers for LDHA (5'-TCTCTGTAGCAGATTTGGCAGA- 3' and 5'- AAGACATCATCCTTTATTCCGTA AAA-3'), MAPK1 (5'-TCTGCACCGTGACCTCAA-3' and 5'- GCCAGGCCAAAGTCACAG-3') and GAPDH (5'- TCCACTGGCGTCTTCACC-3' and 5'- GGCAGAGATGATGACCCTTTT-3') as house-keeping gene.

Animals.

1. Effect of Stiripentol on urine oxalate excretion

Six weeks old Sprague-Dawley male rats were purchased from Harlan Laboratories (France). All efforts were performed to reduce animal suffering. They were housed in similar conditions (3 rats/cage) with a 12-h dark/light cycle and fed ad libitum on standard rat chow. Rats received Stiripentol (Biocodex, France) orally at 200 mg/Kg twice by gavage and urine samples were collected before treatment, 48 hours after treatment initiation and after a wash-out period.

2. Ethylene glycol intoxication protocol

Six 8-weeks old Sprague-Dawley male rats (“control” group) received ethylene glycol by gavage (Batch # SHBG0526V, Sigma Aldrich, France) at 6g/Kg once. Six rats (“Stiripentol” group) received ethylene glycol orally at 6g/Kg and Stiripentol at 300mg/Kg by gavage at the same time. Rats in the “Stiripentol” group had a free access to water containing Stiripentol at 4g/L whereas rats from the control group had a free access to water without Stiripentol. All animals were sacrificed 48 hours later.

3. Oxalate nephropathy protocol.

Sixteen 8-weeks old Sprague-Dawley male rats had a free access to water containing 2g/l calcium (calcium chloride) and 20 g/L hydroxyproline (Batch 090289, Interchim SA, France) during 16 days. Eight of them received Stiripentol at 300 mg/kg by daily gavage, during 16 days.

Environmental enrichment was routinely performed. All animal procedures of the laboratory are performed in accordance with the European Union Guidelines for the Care and Use of laboratory animals and with local Institutional Animal Care and Use Committees (“comité d’éthique en experimentation Charles Darwin C2EA-05”) guidelines.

Biological samples and Biochemistry.

Ethylene glycol intoxication protocol.

A sample of urine has been collected before protocol and at the time of the sacrifice in the acute model of ethylene glycol poisoning to assess crystalluria. A blood serum sample has been collected before protocol and at the time of the sacrifice. The blood samples have been analyzed for creatinine by enzymatic assay, bicarbonate, sodium, chloride and potassium to

assess renal function and acidosis, on an iSYS analyzer from Immunodiagnostic Systems and an ABL815 from Radiometer.

Oxalate nephropathy protocol

Urine has been collected before the protocol and at days 2,4,8,11 and 15 during 24 hours in metabolic cages with free access to water (enriched in calcium and hydroxyproline), to assess urine oxalate excretion. Fresh urine has been collected after spontaneous voiding to perform crystalluria before the protocol and at days 4,8,11 and 15, immediately before 24-hr urine collection. The number, size and type of crystals have been analyzed by trained technicians to measure the mean crystalline volume.¹² Blood (1 mL) has been collected at the time of the sacrifice to measure renal function.

Oxalate urinary excretion in humans

A single urine sample has been collected in patients affected by Dravet syndrome, in Necker Hospital (Paris) after parental information and written consent, ethical committee authorization N° ID-RCB 2016-A01032-49. Urine oxalate excretion in patients affected by cystinuria was obtained from Tenon hospital (Paris) database (CNIL declaration number 1709404 v0), this dosage is routinely performed in all kidney stone formers. Urine oxalate excretion in the patient affected by primary hyperoxaluria has been collected prospectively before and after Stiripentol therapy.

Creatinine has been analyzed by enzymatic methods (blood) and Jaffe method (urine) on an iSYS analyzer from Immunodiagnostic Systems. Oxalate and glycolate urine levels have been measured by ion chromatography (Dionex ICS-3000).

Histology, Yasue staining and morphometry

Kidney tissues were fixed in AFA and formalin and embedded in paraffin. Four- μ m tissue sections have been performed and stained by Yasue procedure to reveal tissue calcifications. A morphometric analysis of calcified tissue surface has been performed with the Image J software (NIH) on 5 photographs at 200x magnification by using polarized light to reveal crystalline deposits.

μ FTIR spectroscopy

Microcalcification phases were characterized using μ Fourier Transform InfraRed spectrometry. Four micrometer tissue sections were deposited on low emission microscope slides (MirrIR, Keveley Technologies, Tienta Sciences, Indianapolis). FT-IR hyperspectral images were recorded with a Spectrum spotlight 400 FT-IR imaging system (Perkin Elmer

Life Sciences, France), with a spatial resolution of 6.25 μm and a spectral resolution of 8 cm^{-1} . The spectra were recorded in the 4000-700 cm^{-1} mid-InfraRed range. Each spectral image, covering a substantial part of the tissue, consisted of about 30,000 spectra.

Statistical analyses. Data are expressed as mean \pm SEM (Figures) and mean \pm SD (Tables). Mann-Whitney and Kruskal Wallis with Dunn's multiple comparison tests were used to compare the different groups, with the exception of serum parameters compared with t-tests (assuming gaussian distribution), by using Prism and Statview softwares. The level of significance was set to < 0.05 .

Study approval

Animal studies: a specific authorization has been obtained from ministry and local ethical committee (N $^{\circ}$ #5110 2016042012069009 v2).

Human studies: ethical committee authorization N $^{\circ}$ ID-RCB 2016-A01032-49.

Authors contributions

EL, MLD and MD designed the study

EL, MLD, SV,ET, EB, RN, NC,GD, LH, JP, CM, JPH, VF, LB, MD conducted experiments

EL, MLD, LH and MD analysed data

EL, MD, MLD and LB wrote the manuscript

Acknowledgements

The authors thank prof. Albert Bensman for his precious help in preparing the final version of the manuscript. This work has been supported by the Agence Nationale de la Recherche (ANR-13-JSV1-0010-01), the Académie Nationale de Médecine (Nestlé-Waters award), Convergence-UPMC CVG1205 and CORDDIM-2013-COD130042.

References

1. Khan SR, Pearle MS, Robertson WG, et al. Kidney stones. *Nat Rev Dis Primer*. 2016;2:16008. doi:10.1038/nrdp.2016.8.
2. Kruse JA. Methanol and ethylene glycol intoxication. *Crit Care Clin*. 2012;28(4):661-711. doi:10.1016/j.ccc.2012.07.002.
3. Cochat P, Rumsby G. Primary hyperoxaluria. *N Engl J Med*. 2013;369(7):649-658. doi:10.1056/NEJMra1301564.
4. Holmes RP, Assimos DG. Glyoxylate synthesis, and its modulation and influence on oxalate synthesis. *J Urol*. 1998;160(5):1617-1624.
5. Sada N, Lee S, Katsu T, Otsuki T, Inoue T. Epilepsy treatment. Targeting LDH enzymes with a stiripentol analog to treat epilepsy. *Science*. 2015;347(6228):1362-1367. doi:10.1126/science.aaa1299.
6. Verrotti A, Prezioso G, Stagi S, Paolino MC, Parisi P. Pharmacological considerations in the use of stiripentol for the treatment of epilepsy. *Expert Opin Drug Metab Toxicol*. 2016;12(3):345-352. doi:10.1517/17425255.2016.1145657.
7. Baker PRS, Cramer SD, Kennedy M, Assimos DG, Holmes RP. Glycolate and glyoxylate metabolism in HepG2 cells. *Am J Physiol Cell Physiol*. 2004;287(5):C1359-1365. doi:10.1152/ajpcell.00238.2004.
8. Farwell JR, Anderson GD, Kerr BM, Tor JA, Levy RH. Stiripentol in atypical absence seizures in children: an open trial. *Epilepsia*. 1993;34(2):305-311.
9. Brent J, McMartin K, Phillips S, et al. Fomepizole for the treatment of ethylene glycol poisoning. Methylpyrazole for Toxic Alcohols Study Group. *N Engl J Med*. 1999;340(11):832-838. doi:10.1056/NEJM199903183401102.
10. Bergstralh EJ, Monico CG, Lieske JC, et al. Transplantation outcomes in primary hyperoxaluria. *Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg*. 2010;10(11):2493-2501. doi:10.1111/j.1600-6143.2010.03271.x.
11. Khan SR, Glenton PA, Byer KJ. Modeling of hyperoxaluric calcium oxalate nephrolithiasis: experimental induction of hyperoxaluria by hydroxy-L-proline. *Kidney Int*. 2006;70(5):914-923. doi:10.1038/sj.ki.5001699.
12. Daudon M, Hennequin C, Boujelben G, Lacour B, Jungers P. Serial crystalluria determination and the risk of recurrence in calcium stone formers. *Kidney Int*. 2005;67(5):1934-1943. doi:10.1111/j.1523-1755.2005.00292.x.

Table

Table I: serum parameters after ethylene glycol intoxication

Serum parameters	EG	EG+ Stiripentol	<i>p</i> value
Creatinine $\mu\text{mol/L}$	297.1 \pm 166.3	78.1 \pm 21.4	0.009
Bicarbonate mmol/L	17.7 \pm 4.15	20.8 \pm 5.6	0.15
Potassium mmol/L	6.5 \pm 2.4	4.8 \pm 1.3	0.07
Sodium mmol/L	137.6 \pm 4.7	147.4 \pm 3.7	<0.001
Chloride mmol/L	93 \pm 7.9	107.6 \pm 9.3	<0.001
Serum Anion Gap mmol/L	33.5 \pm 11.1	23.7 \pm 12.2	0.04

Serum parameters at day 2 after ethylene glycol intoxication (n=6 animals/group). Results are Data are expressed as mean \pm SD. Student t-test (assuming gaussian distribution of biological values) were performed. EG= ethylene glycol

Figure legends

Figure 1: Inhibition of oxalate synthesis by Stiripentol in vitro and in vivo. HepG2 cells were grown in a hydroxyproline-enriched medium to produce oxalate (red bars). Oxalate synthesis (mM) was reduced in a dose-dependent manner when Stiripentol was added to the medium (Figure 1A *: $p=0.03$, $n=4$ experiments). SiRNA targeting LDH-5 subunits reduced significantly oxalate synthesis and the addition of $10\mu\text{g/mL}$ Stiripentol to SiRNA reduced mildly oxalate synthesis, suggesting that oxalate synthesis is mostly performed by LDH-5 (Figure 1B *: $p=0.03$ vs control, $n=4$ experiments). Stiripentol given orally during 2 days reduced significantly urine oxalate excretion (mmol/mmol: mmol oxalate/mmol creatinine, Figure 1 C *: $p=0.002$, $n=6$ animals). After wash-out, urine oxalate excretion was restored. Data are expressed as mean \pm SEM. Mann-Whitney tests were used to compare the different groups.

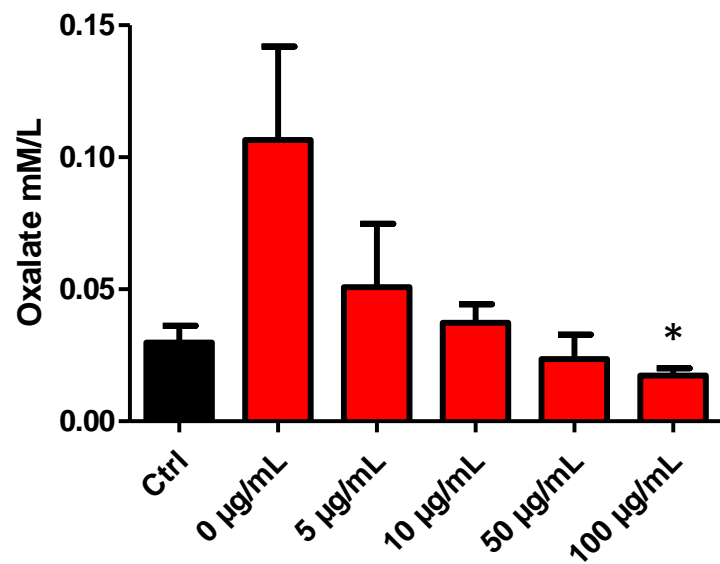
Figure 2: Stiripentol protects against ethylene glycol intoxication. Representative crystalluria evidencing the presence of calcium oxalate monohydrate crystals (COM, black arrow) and to a lesser extent calcium oxalate dihydrate crystals (COD, white arrow) in urine (Figure 2A). Mean crystalline volume in urine was significantly lower in animals receiving Stiripentol in addition to ethylene glycol (EG + Stiripentol: ■) than in animals receiving ethylene glycol alone (EG: ●) (Figure 2B *: $p=0.004$, $n=6$ animals/group). The weight of kidneys from rats exposed to ethylene glycol only was increased when compared to kidneys from rats treated by Stiripentol (Figure 2C, *: $p=0.004$, $n=6$). Kidney crystalline accumulation was prevented by Stiripentol (Figures 2D-F, *: $p=0.002$, $n=6$). Stiripentol protected rats against ethylene-glycol-induced renal failure ($p=0.002$, Figure 2G, $n=6$). Crystalline deposits were stained by Yasue coloration evidencing their calcic nature (Figure 2H) and Fourier transform infrared spectroscopy (FTIR) revealed the exclusive presence of COM among tubular deposits (Figure 2I). Data are expressed as mean \pm SEM. Mann-Whitney and Kruskal Wallis with Dunn's multiple comparison tests were used to compare the different groups.

Figure 3: Stiripentol protects against calcium oxalate nephropathy. Urine oxalate excretion (indexed to creatinine, mmol/mmol) was increased by hydroxyproline (HP)-enriched diet and the daily administration of Stiripentol (red bars) protected partly against hyperoxaluria (Figure 3A, $p=0.33$ at day 2, $p=0.10$ at day 4, *: $p=0.0002$ at day 8, *: $p=0.0019$ at day 11, *: $p=0.0002$ at day 15, $n=8$ animals/group) and reduced urine crystalline volume (Figure 3B, $p=0.15$ at day 4, *: $p=0.015$ at day 8, $p=0.08$ at day 11, *: $p=0.007$ at day 15, $n=8$ animals/group). Kidney crystal deposits were reduced by Stiripentol (HP + Stiripentol: ■, HP: ●) (Figures 3C-E *: $p=0.004$, $n=8$ animals/group). Stiripentol protected rats against hydroxyproline-induced renal failure (Figure 3F, *: $p=0.028$, $n=8$ animals/group). Data are expressed as mean \pm SEM. Mann-Whitney and Kruskal Wallis with Dunn's multiple comparison tests were used to compare the different groups

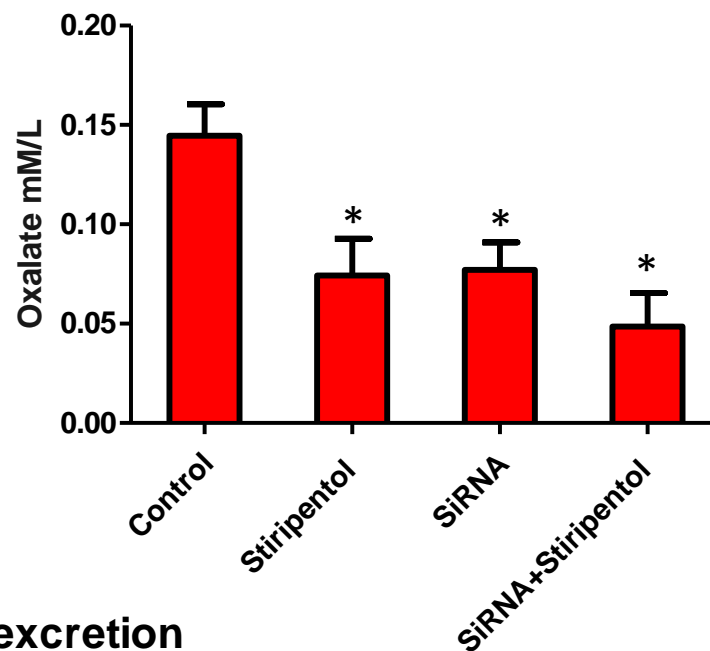
Figure 4: Stiripentol decreases urine oxalate excretion in humans. Urine oxalate excretion (indexed to creatinine, mmol/mmol) was lower in children affected by Dravet syndrome and treated with Stiripentol than in controls, i.e. children affected by cystinuria (Figure 4A, $p=0.002$; Dravet + Stiripentol: ■, $n=40$; Controls: ●, $n=8$). Mann-Whitney test was used to compare the two groups. Urine oxalate excretion (indexed to creatinine, mmol/mmol) has been measured before and after Stiripentol therapy (25 mg/Kg/day and 50 mg/Kg/day) in a young girl affected by primary hyperoxaluria type I.

Supplementary Figure 1: Urine glycolate excretion (indexed to creatinine, mol/mmol) was increased by hydroxyproline (HP)-enriched diet in rats. At day 2 and day 8 glycolate excretion was significantly more increased in animals exposed to Stiripentol than in controls ($*p=0.049$ and $p=0.004$ respectively, $n=8$ animals/group). Data are expressed as mean \pm SEM. Mann-Whitney and Kruskal Wallis with Dunn's multiple comparison tests were used to compare the different groups.

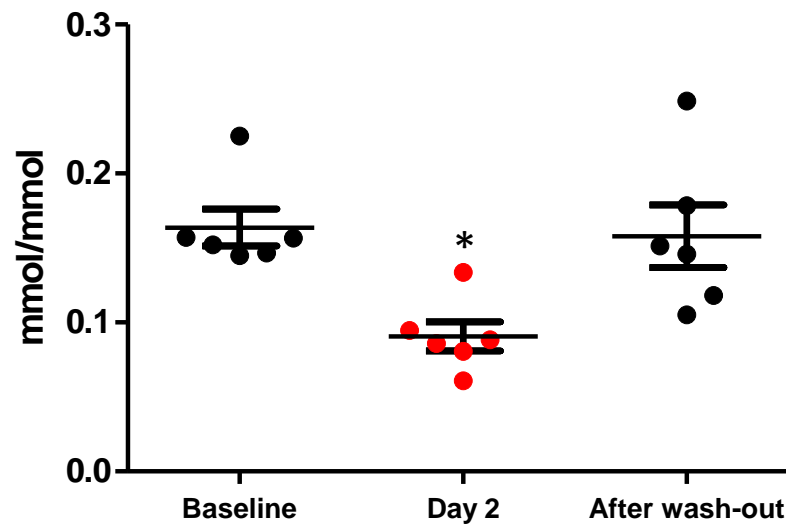
A Oxalate synthesis by hepatocytes

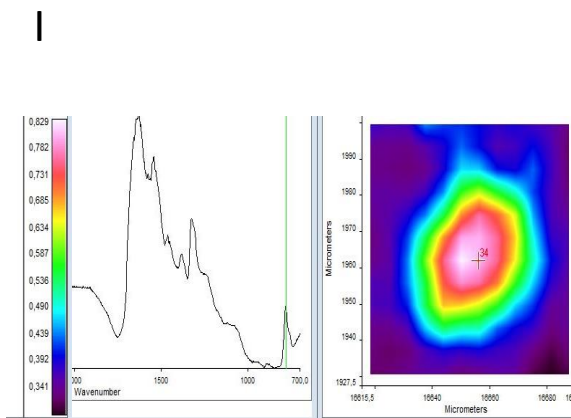
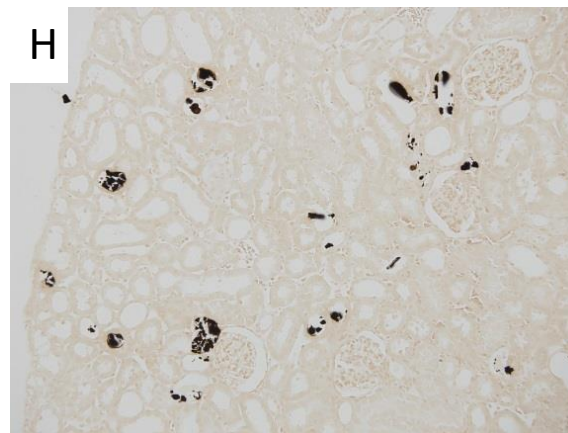
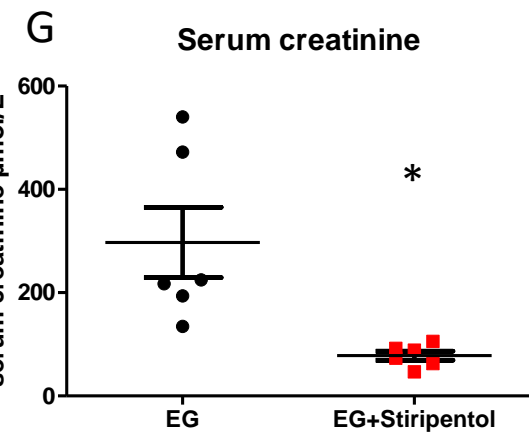
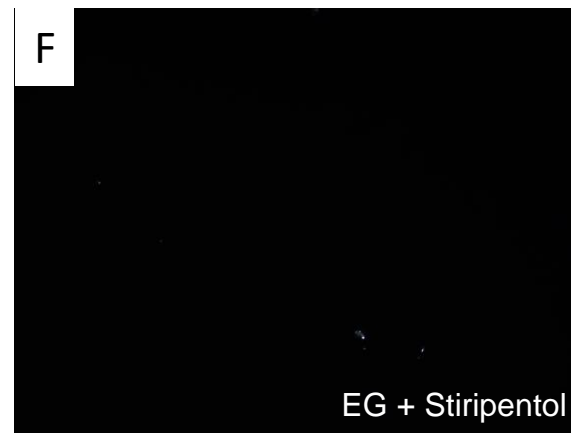
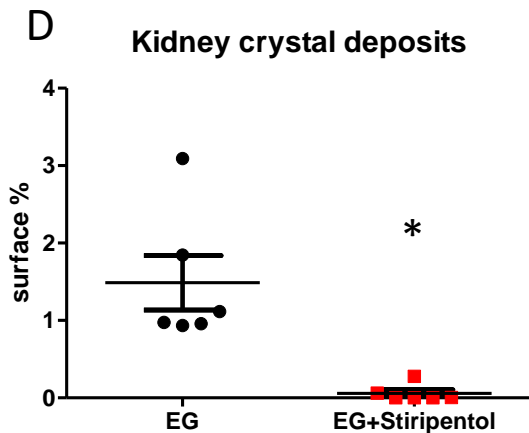
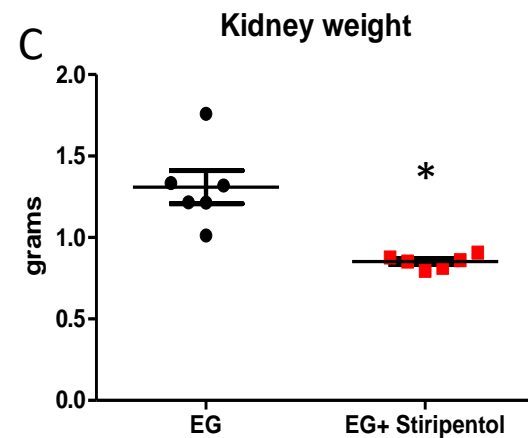
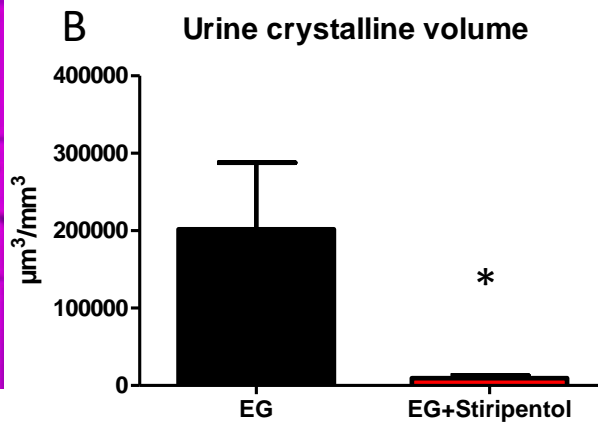
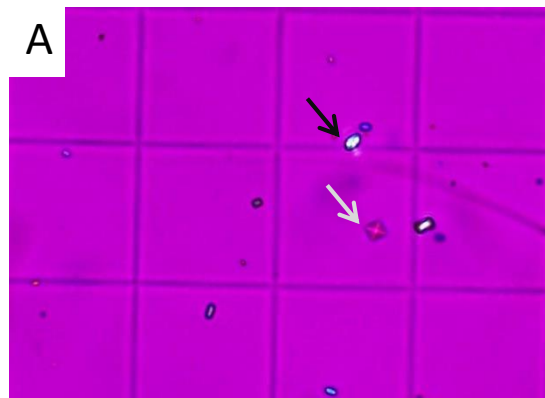


B Oxalate synthesis by hepatocytes

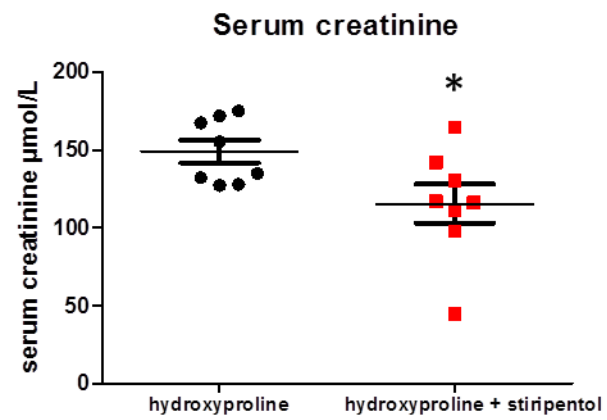


C Urine oxalate excretion



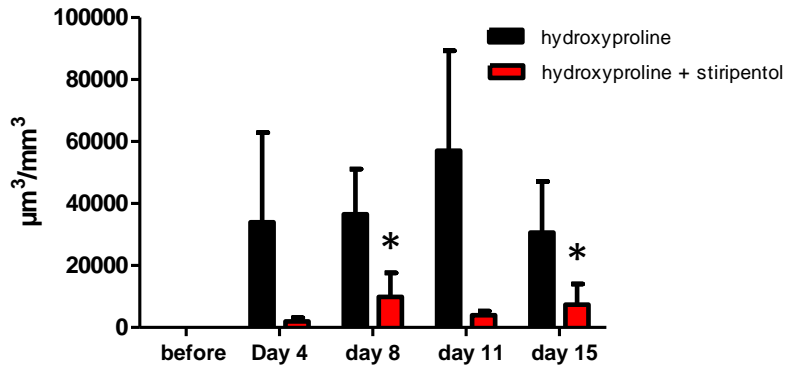


* * *



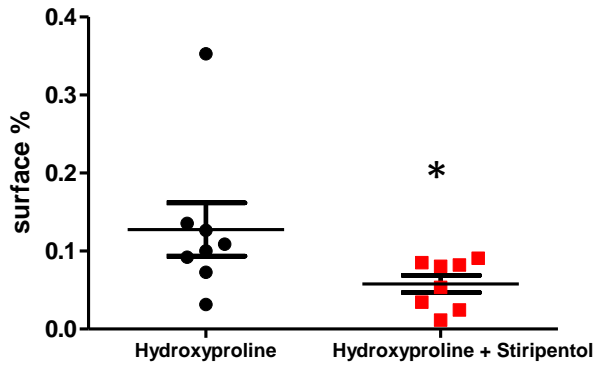
A

Urine crystalline volume

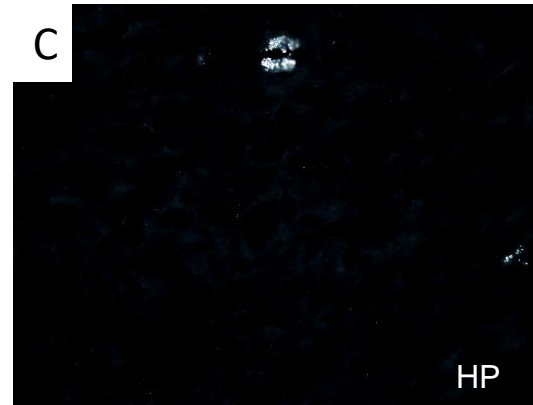


B

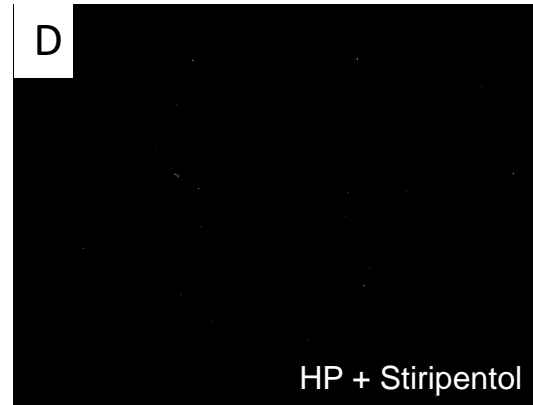
Kidney crystal deposits



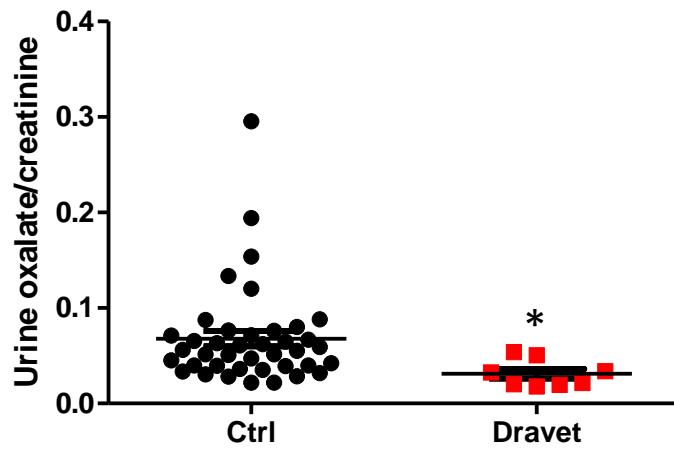
C



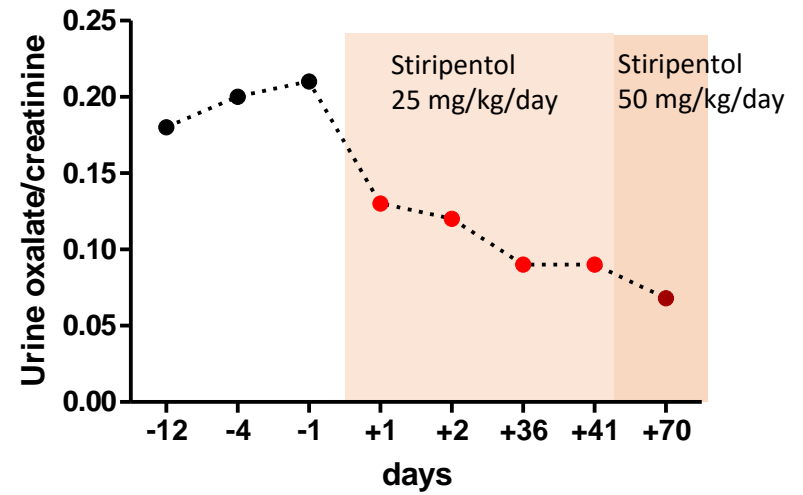
D



A urine oxalate excretion in Dravet patients



B Stiripentol therapy



Urine glycolate excretion

