CASE REPORT

REVISED Case Report: Diabetic ketoacidosis after co-administration of empagliflozin and probenecid [version 2; peer review: 2 approved, 1 approved with reservations]

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Abstract

Sodium-glucose cotransporter-2 (SGLT2) inhibitors are filtered and secreted to their primary site of action in the proximal tubule of the kidney. At this site, SGLT2 inhibitors also reduce renal elimination of ketone bodies, a finding implicated in their propensity to cause ketoacidosis. Many commonly used medications have potential to diminish renal elimination of SGLT2 inhibitors and to compound the effects of SGLT2 inhibitors on renal elimination of ketone bodies by inhibiting tubular secretion of the SGLT2 inhibitor itself and/or ketone bodies. We present a case of severe diabetic ketoacidosis (DKA) in a patient with type 2 diabetes occurring several days after co-prescription of empagliflozin and probenecid. Other than the recent introduction of empagliflozin, no cause for the DKA episode was apparent. A pharmacokinetic interaction between probenecid and empagliflozin, involving organic anion transporter 3 (OAT3), reduces proximal tubular secretion of empagliflozin and increases patient exposure to the drug. Whether or not this phenomenon is sufficient to cause severe DKA is discussed. An alternative explanation as to the DKA aetiology is proposed, wherein probenecid may compound effects of empagliflozin on renal elimination of ketone bodies. We suggest that clinicians exercise caution when prescribing SGLT2 inhibitors alongside pharmacologic inhibitors of, or competitors for, proximal tubular organic anion transporters in patients with diabetes mellitus due to the risk of severe DKA.

Open Peer Review

Approval Status ✓ ? ✓

	1	2	3
version 2 (revision) 05 Mar 2024	✓ view		✓ view
version 1 21 Jun 2023	? view	? view	

1. **Simeon I Taylor**, University of Maryland School of Medicine, Baltimore, USA
Zhinous Shahidzadeh Yazdi, University of Maryland Baltimore, Baltimore, USA
University of Maryland School of Medicine, Baltimore, USA
2. **Prashant Nasa**, Max Super Specialty Hospital Saket, New Delhi, India
3. **Francis M Finucane**, University of Galway, Galway, Ireland

Any reports and responses or comments on the article can be found at the end of the article.

Keywords
diabetic ketoacidosis, DKA, empagliflozin, SGLT2 inhibitor, SGLT2i, probenecid, organic anion transporter 3, OAT3, case report

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Author roles: Martin WP: Conceptualization, Funding Acquisition, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Reidy N**: Writing – Review & Editing; **Low J**: Writing – Review & Editing; **Ahern T**: Conceptualization, Writing – Original Draft Preparation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

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First published: 21 Jun 2023, **8**:268 https://doi.org/10.12688/wellcomeopenres.19148.1

REVISED **Amendments from Version 1**

Thank you to the reviewers for their thorough review of this case report and constructive feedback. In this revised version, we have responded to the reviewer feedback by:

- Updating Figure 1 by removing extraneous arrows to improve its clarity;
- Discussing mechanisms of diabetic ketoacidosis in the setting of SGLT2 inhibitor therapy;
- Highlighting the lack of plasma empagliflozin levels before and after probenecid administration as a limitation of the case report;
- Providing more detailed discussion of the pharmacokinetic drug-drug interaction between empagliflozin and probenecid, and whether or not increased systemic exposure to empagliflozin alone would be sufficient explain the severe episode of diabetic ketoacidosis observed;
- Proposing an alternative or complementary explanation for the episode of diabetic ketoacidosis arising due to the empagliflozin-probenecid drug-drug interaction, wherein both drugs act to diminish renal elimination of ketone bodies; and
- Expanding the conclusions of the article to highlight the potential for ketoacidosis with co-prescription of SGLT2 inhibitors and inhibitors of proximal tubular organic anion transporters, as well as making recommendations for future studies to tease out the mechanisms involved in the generation of ketoacidosis in this context.

Any further responses from the reviewers can be found at the end of the article

Introduction

Sodium-glucose cotransporter-2 (SGLT2) inhibitors are strongly recommended for patients with type 2 diabetes in the latest clinical practice guidelines based on their demonstrated cardiovascular and renal benefits[1]. Nevertheless, these medications increase the risk of diabetic ketoacidosis (DKA)[2,3], a severe acute complication of diabetes which carries a substantial risk of morbidity and mortality[4].

SGLT2 inhibitors are both filtered and secreted to their primary site of action in the proximal tubule of the kidney[5]. Although many commonly prescribed medications have the potential to diminish renal elimination of SGLT2 inhibitors by inhibiting their proximal tubular secretion, these interactions are not recognised to increase the risk of DKA nor other side effects of SGLT2 inhibitors and do not influence routine prescription of these medications. We present a case of severe DKA in a patient with type 2 diabetes occurring several days after co-prescription of the SGLT2 inhibitor empagliflozin alongside probenecid, an inhibitor of organic anion transporter 3 (OAT3)[6], a transporter which plays a key role in the secretion of empagliflozin into the proximal tubule[5].

Case report

A 54-year-old woman with obesity (body-mass index 39.5 kg/m^2) and type 2 diabetes mellitus (T2DM, diagnosed 7 years ago) presented to our hospital with a one-week history of right leg redness, swelling, and pain (Figure 1). There was a family history of type 1 diabetes mellitus (T1DM) diagnosed at 8 years of age in a maternal first cousin and of T2DM diagnosed in a maternal uncle in his mid-50s. The patient lived alone, was independent in activities of daily living, drank alcohol rarely on social occasions, and was a lifelong non-smoker. On examination, the patient had a heart rate of 118 beats per minute (sinus tachycardia on an electrocardiogram), respiratory rate of 19 breaths per minute, blood pressure of 141/91 mmHg, peripheral oxygen saturations of 100% on room air, and a temperature of 38.8 degrees Celsius. Erythema, swelling, and tenderness of the entire right lower limb was noted. Examination of the cardiovascular, respiratory, and abdominal systems was unremarkable.

Ultrasound imaging confirmed the presence of a right iliofemoral deep vein thrombosis. The blood glycosylated haemoglobin level was 107 mmol/mol. *Streptococcus agalactiae* was cultured in peripheral blood samples. No evidence of infective endocarditis was present on transthoracic echocardiography. The patient was anticoagulated with enoxaparin 1 mg/kg (90 mg) twice daily subcutaneously initially, and later transitioned to apixaban 5 mg twice daily orally. Metformin therapy was continued at a dose of 850 mg three times daily orally, modified release gliclazide 120 mg once daily orally was stopped, and linagliptin 5 mg once daily orally and empagliflozin 10 mg once daily orally were commenced to improve glycaemic control.

The *Streptococcus agalactiae* isolate tested susceptible to penicillin, and treatment with intravenous high dose benzylpenicillin 2.4 g four times daily was commenced with good clinical effect. The source of bacteraemia was unclear; however, given the presence of a deep vein thrombosis, this was presumed to be infected. Blood C-reactive protein and neutrophil count values decreased during her inpatient stay (196 to 33 mg/L and 10.6 to 5.7 x10^9/L, respectively). Prior to discharge, a peripherally inserted central catheter was placed and the antibiotic regimen was switched to intravenous cefazolin (2 g once daily) with oral probenecid (1g once daily) to facilitate a 6-week antibiotic course via the outpatient parenteral antibiotic therapy service.

The patient was readmitted 48 hours later with severe DKA. The patient's infection was well controlled at the point of readmission with DKA. The patient had received the prescribed cefazolin via the outpatient parenteral antibiotic therapy service after hospital discharge. The patient also reported good compliance with prescribed medications, including oral hypoglycaemics, after hospital discharge. Nevertheless, venous blood gas results included: pH 6.87; bicarbonate 3.6 mmol/L; anion gap 30.4 mmol/L; and glucose 15.8 mmol/L. The blood ketone level was 4.3 mmol/L. The patient was treated with a variable rate intravenous insulin infusion, which was prepared by adding 50 units of Actrapid human soluble insulin to 49.5 mL of 0.9% sodium chloride and administered at insulin infusion rates of 0.5 - 8mL/hour. Intravenous isotonic sodium bicarbonate 1.26%, balanced crystalloid (Hartmann's

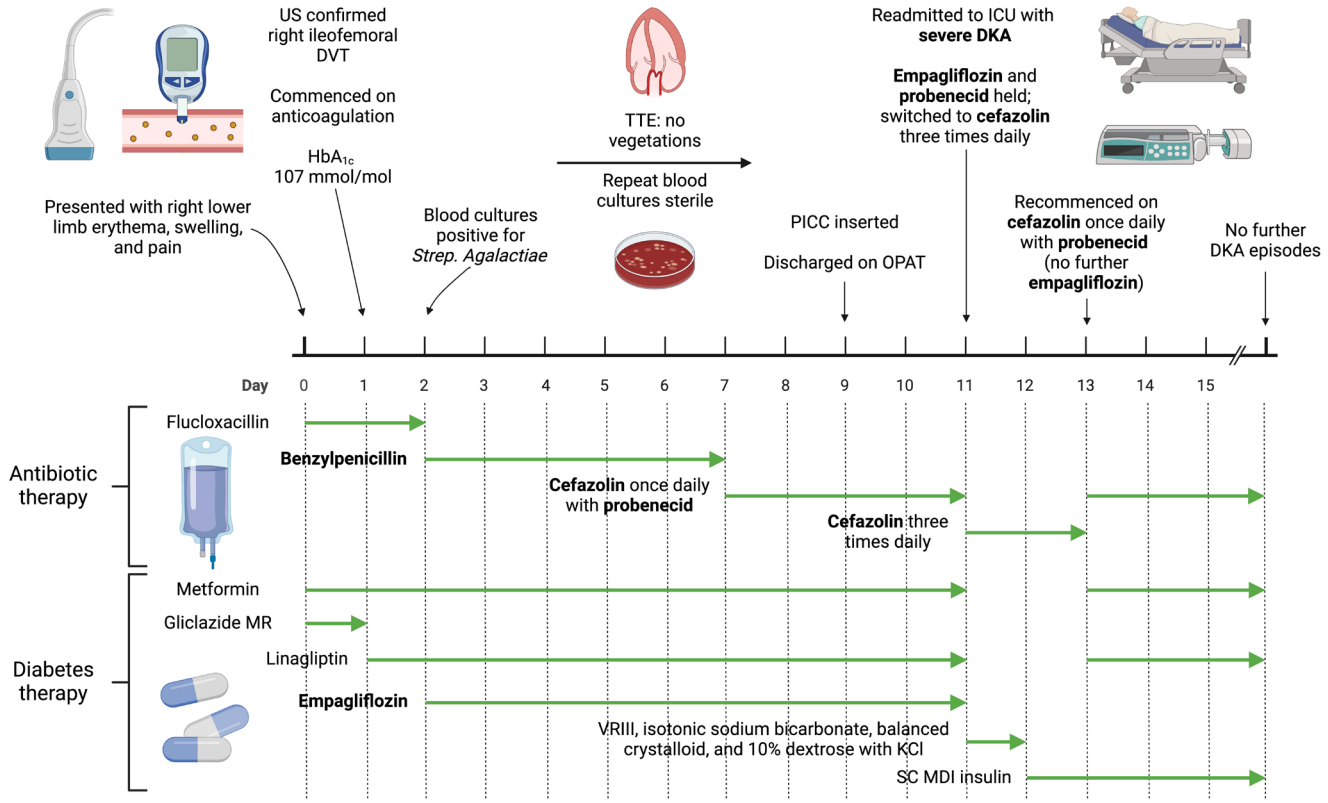

Figure 1. Overview of patient presentation and management, outlining timing of emergence of diabetic ketoacidosis after co-administration of empagliflozin and probenecid. Created with BioRender.com. DKA, diabetic ketoacidosis; DVT, deep vein thrombosis; HbA_{1c}, glycated haemoglobin; ICU, intensive care unit; KCl, potassium chloride; MDI, multiple daily injection; MR, modified release; OPAT, outpatient parenteral antibiotic therapy; PICC, peripherally inserted central catheter; SC, subcutaneous; TTE, transthoracic echocardiogram; VRIII, variable rate intravenous insulin infusion. Figure 1 was created with BioRender.com.

solution), and 10% dextrose with potassium chloride were also administered as per institutional protocol. Subcutaneous insulin therapy was commenced when the DKA resolved: insulin detemir (Levemir®) 10 units twice daily and insulin aspart (Fiasp®) 8 units three times daily with meals. Metformin 500 mg twice daily orally and linagliptin 5 mg once daily orally were recommenced 48 hours after readmission, with the metformin uptitrated to 1 g twice daily after one week.

At the point of readmission with severe DKA, and after having commenced systemic anticoagulation for the deep venous thrombosis, the patient reported vaginal bleeding and an ulcerated vulval lesion was noted on physical examination. Biopsies later confirmed the presence of a squamous cell carcinoma, which was staged as IVB disease on the basis of inguinal and pelvic sidewall lymphadenopathy and bone metastases present on staging scans. The vulval cancer is likely to have contributed to formation of the deep vein thrombosis as well as serving as a source of entry of *Streptococcus agalactiae* into the bloodstream[7].

Discussion

Other than the introduction of empagliflozin 9 days prior, no cause for the severe DKA episode described in this case was apparent. SGLT2 inhibitors are recognised to increase risk of DKA. A meta-analysis of 39 randomised clinical trials highlighted that SGLT2 inhibitors double the risk of DKA in people with T2DM[2]. This risk is higher in elderly patients and those using SGLT2 inhibitors for longer periods of time[2], neither of which applied in the current case. Whilst SGLT2 inhibitors substantially increase risk of DKA in patients with T1DM[3], further evaluation for T1DM after readmission with DKA was negative in the present case. Specifically, an islet autoantibody screen including antibodies to zinc transporter 8, islet antigen 2, and glutamic acid decarboxylase was negative and the 2-hour post-prandial urinary C-peptide to creatinine ratio was 3.51 nmol/mmol, indicative of substantial endogenous insulin secretion.

Apart from undiagnosed T1DM, other common precipitants of SGLT2 inhibitor-induced DKA include infection, surgery, prolonged fasting, alcohol intake, acute vascular events (such as acute coronary syndrome or stroke), trauma, and prolonged exercise[8]. Of these, only infection is potentially pertinent to the DKA episode described herein. However, the patient's infection was well controlled at the time of readmission with DKA. Thus, the occurrence of DKA within several days of co-prescription of empagliflozin and probenecid implicates

interaction between these medications rather than other factors as the primary cause of the severe DKA observed in this case.

Empagliflozin reaches its primary site of action in the proximal tubule by a combination of glomerular filtration and tubular secretion by transporters including OAT3, which is expressed on the basolateral membrane of the proximal tubule[6]. Probenecid inhibits OAT3[6], with this property underlying its use alongside cefazolin in the present case. By diminishing renal cefazolin secretion, sufficient circulating cefazolin concentrations are achieved with once daily dosing.

In a pharmacokinetic study in healthy volunteers, co-administration of probenecid and empagliflozin, at the same total daily doses as those in the present case (1 g and 10 mg, respectively), resulted in a 26% increase in peak empagliflozin plasma concentrations and a 53% increase in area under the concentration-time curve[6]. These findings were not felt to be clinically meaningful[6]. However, the impact of this pharmacokinetic interaction between empagliflozin and probenecid has not been studied in patients with T2DM and it is unclear to what extent empagliflozin exposure would increase in the presence of additional OAT3 inhibitors alongside probenecid. In the present case, co-administration of empagliflozin and probenecid immediately preceded a presentation with DKA with no other apparent cause. Moreover, both benzylpenicillin and cefazolin, antibiotics administered to this patient while she was receiving empagliflozin, are OAT3 inhibitors and likely contributed to enhanced empagliflozin exposure[9,10]. Although not routinely available in clinical practice, the absence of plasma empagliflozin levels before and after administration of probenecid is a limitation of this case report. In the absence of drug levels confirming increased systemic exposure to empagliflozin, we can only associate rather than definitively implicate co-prescription of empagliflozin and probenecid in the aetiology of the severe DKA described herein.

Although a plausible hypothesis, certain lines of evidence argue against increased systemic exposure to empagliflozin alone being sufficient to cause the severe DKA described herein. Even if systemic exposure to empagliflozin increased by 53% with probenecid[6], this would not be expected to exceed the typical systemic exposure with the higher approved dose of empagliflozin (25 mg daily) - which does not routinely cause ketoacidosis. Although increased glucosuria is implicated in the mechanism of ketoacidosis with SGLT2 inhibitors[8,11], the clinically approved doses of SGLT2 inhibitors (10 and 25 mg daily in the case of empagliflozin) were selected because they are near the plateau of the dose-response curve for glucosuria and are believed to achieve near-total inhibition of SGLT2[12,13]. Thus, even with increased systemic exposure to empagliflozin due to probenecid, one would not expect a marked increase in the magnitude of glucosuria. Moreover, mice with genetic knockout of OAT3 had a diminished glucosuric response to empagliflozin despite comparable tubular secretion of the drug to wild-type controls[5]. Although the physiology may differ between rodents and humans, it seems unlikely that increased glucosuria resulting in increased ketogenesis was the sole driver of ketoacidosis in the present case.

The severe DKA episode described herein may be better explained by the effects of SGLT2 inhibitors and probenecid on renal excretion of ketone bodies. Phlorizin (a non-selective SGLT1/SGLT2 inhibitor and the chemical structure upon which empagliflozin is based) increased renal tubular reabsorption of acetoacetate in dogs[11,14]. Through mechanisms reviewed in greater detail by Taylor *et al*., phlorizin would be expected to exert a similar effect on renal tubular handling of β-hydrobutyrate[11]. It is therefore plausible that empagliflozin decreases urinary excretion of ketone bodies, an effect which was compounded by co-administration of probenecid in the present case. Probenecid inhibits the organic anion transporters, OAT1 and OAT3[6,15]. Knockout of the Oat1 gene in mice decreased urinary excretion of the ketone body 3-hydroxybutyrate by approximately 65% and dramatically elevated plasma concentrations of 3-hydroxybutyrate, suggesting that 3-hydroxybutyrate is a physiological substrate of OAT1[16]. The ligand specificity of OAT1 and OAT3 also appears to be overlapping[17]. This raises the possibility that the severe DKA in this patient arose due to reduced urinary excretion of ketone bodies caused by concurrent treatment with probenecid and other inhibitors of renal organic anion transporters, including benzylpenicillin and cefazolin[9,10], alongside the SGLT2 inhibitor empagliflozin. Of course, other factors implicated in ketoacidosis caused by SGLT2 inhibitors, such as increased ketogenesis as a consequence of glucosuria as well as increased glucagon secretion would have served to exacerbate the ketoacidosis[8,11].

In conclusion, we suggest that clinicians exercise caution when co-prescribing empagliflozin and probenecid, or indeed other inhibitors of proximal tubular organic anion transporters, which may increase systemic exposure to the SGLT2 inhibitor and/or diminish urinary excretion of ketone bodies resulting in ketoacidosis. A similar drug-drug interaction may be relevant to dapagliflozin, which is also a substrate of OAT3[18]. Ketoacidosis arising in this context may be severe. As co-prescription of SGLT2 inhibitors alongside inhibitors of proximal tubular organic anion transporters is likely to increase, investigation into the mechanisms of ketoacidosis in this context warrant further study. Studies assessing plasma levels of SGLT2 inhibitors and ketone bodies (β-hydroxybutyrate, acetoacetate, and acetone) before and after co-prescription of SGLT2 inhibitors with inhibitors of proximal tubular organic anion transporters in healthy volunteers and in people with diabetes mellitus would be informative.

Consent

Written informed consent for publication of their clinical details was obtained from the patient.

Data availability

All data underlying the results are available as part of the article and no additional source data are required.

Reporting guidelines

Open Science Framework: CARE checklist for 'Case Report: Diabetic ketoacidosis after co-administration of empagliflozin and probenecid'. https://doi.org/10.17605/OSF.IO/8VJEQ

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Open Peer Review

Current Peer Review Status: ✔ ? ✔

Version 2

Reviewer Report 06 August 2024

https://doi.org/10.21956/wellcomeopenres.23373.r89611

✔ **Francis M Finucane**
Galway University Hospitals, University of Galway, Galway, Ireland

This is an excellent description of an interesting case which makes an important contribution to the literature about a challenging and still poorly understood clinical problem. The previous reviewer comments are insightful and constructive and the responses are comprehensive. The figure is very clear and helpful.

I would have the following minor comments about the latest version of the case report:

I would specify the ethnicity of the patient at the start as it is always relevant in considering diabetes phenotype, especially in DKA occurring in type 2 diabetes.

An excellent overview of the mechanistic basis for SGLT2i induced DKA was published last year by Fahy et al. (2023[1]) and would be worth incorporating and referencing (PMID 37261785).

It would be worth giving some specific examples when it is first mentioned that other drugs can inhibit OAT.

It would be good to provide more clarity and specificity around the epidemiology of SGLT2i DKA - some papers suggesting they do more than just "doubling" the risk of DKA in patients with T2DM.

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Is the background of the case's history and progression described in sufficient detail?
Yes

Are enough details provided of any physical examination and diagnostic tests, treatment given and outcomes?

Partly

Is sufficient discussion included of the importance of the findings and their relevance to future understanding of disease processes, diagnosis or treatment?
Yes

Is the case presented with sufficient detail to be useful for other practitioners?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Obesity, type 2 diabetes, ketone metabolism.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 05 April 2024

https://doi.org/10.21956/wellcomeopenres.23373.r75983

Simeon I Taylor
University of Maryland School of Medicine, Baltimore, USA
Zhinous Shahidzadeh Yazdi
[1] Medicine, University of Maryland Baltimore, Baltimore, Maryland, USA
[2] Medicine, University of Maryland School of Medicine, Baltimore, Maryland, USA

The revised version fully addresses the comments in our review and is a valuable contribution to the literature.

Is the background of the case's history and progression described in sufficient detail?
Yes

Are enough details provided of any physical examination and diagnostic tests, treatment given and outcomes?
Yes

Is sufficient discussion included of the importance of the findings and their relevance to future understanding of disease processes, diagnosis or treatment?
Yes

Is the case presented with sufficient detail to be useful for other practitioners?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: I am a physician-scientist with a special interest in endocrinology and diabetes -- especially with respect to drugs to treat diabetes and obesity. Based on my work at Bristol-Myers Squibb where I participated in R&D leading to approval of dapagliflozin, I have specialized expertise related to SGLT2 inhibitors.

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 16 February 2024

https://doi.org/10.21956/wellcomeopenres.21226.r72788

?

Prashant Nasa
Max Super Specialty Hospital Saket, New Delhi, India

Thank you for presenting this interesting case of DKA with the use of SGLT2 inhibitors, empagliflozin in this case. The case history is very well presented through a graphical abstract.

- However, the use of two coloured arrows to depict the drugs creates confusion. It would be better to use a single arrow for the start and end of the drug.
- The conclusion is missing from the article.
- The mechanisms described for DKA with the use of SGLT-2 inhibitors are multifactorial. I recommend using this review article to discuss this in detail [1].
- Since the drug levels of empagliflozin were not measured in this case, the causal relationship between empagliflozin and probenecid drug interactions could not be established.
- I believe this should be included as a limitation.
- In addition, include a discussion on other possible mechanisms of DKA related to SGLT-2 inhibitors in this case.

References

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Is the background of the case's history and progression described in sufficient detail?

Yes

Are enough details provided of any physical examination and diagnostic tests, treatment given and outcomes?
Yes

Is sufficient discussion included of the importance of the findings and their relevance to future understanding of disease processes, diagnosis or treatment?
No

Is the case presented with sufficient detail to be useful for other practitioners?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Intensive Care Medicine

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 20 Feb 2024

William Martin

Thank you for your thorough review of this case report and constructive feedback. We have responded to your comments by:

- Updating Figure 1 by removing extraneous arrows to improve its clarity;
- Discussing mechanisms of diabetic ketoacidosis in the setting of SGLT2 inhibitor therapy;
- Highlighting the lack of plasma empagliflozin levels before and after probenecid administration as a limitation of the case report;
- Expanding the conclusions of the article to highlight the potential for ketoacidosis with co-prescription of SGLT2 inhibitors and inhibitors of proximal tubular organic anion transporters, as well as making recommendations for future studies to tease out the mechanisms involved in the generation of ketoacidosis in this context.

Competing Interests: No competing interests were disclosed.

Reviewer Report 13 October 2023

https://doi.org/10.21956/wellcomeopenres.21226.r68333

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Martin et al. have provided an astute description of a thought-provoking case of ketoacidosis in a patient with type 2 diabetes treated with a multi-drug regimen including empagliflozin as well as probenecid. Because of the timing of ketoacidosis shortly after addition of probenecid to the therapeutic regimen, the authors hypothesize that a pharmacokinetic drug-drug interaction between empagliflozin and probenecid may have contributed to the pathogenesis of ketoacidosis. Although based on circumstantial evidence, this is a reasonable hypothesis. Specifically, the authors hypothesize that probenecid decreases organic anion transporter (OAT)-mediated renal excretion of empagliflozin. As discussed below, the literature contains substantial evidence that does not fully support their specific mechanistic hypothesis. It would have been helpful if the authors had provided data on plasma levels of empagliflozin before and after addition of probenecid. However, these analyses are not usually measured in routine clinical practice. In our review of the manuscript, we propose an alternative mechanism that might mediate a pharmacodynamic drug-drug interaction – specifically a possible role for organic anion transporters in mediating renal excretion of ketone bodies. The case report would be strengthened by adding a discussion of this alternative mechanism.

SPECIFIC COMMENTS

1. Pharmacokinetic drug-drug interaction. Mach et al. (Clin Ther, 36, 280, 2014) [reference #6 in the paper by Martin et al.] reported formal drug-drug interaction studies indicating that probenecid increased exposure to empagliflozin by 53% (90% confidence interval, 46-61%). However, the patient was receiving the 10 mg/day dose of empagliflozin. Even if the exposure increased by 61%, the exposure would not be predicted to exceed the range of exposures typically observed in patients receiving the higher approved dose of empagliflozin (25 mg/day). Thus, it seems unlikely that the relatively modest increase in empagliflozin exposure does not seem to be sufficient to account for the patient's ketoacidosis. Furthermore, the approved doses of SGLT2 inhibitors were selected because they are near the plateau of the dose-response curve for glucosuria and are believed to achieve near-total inhibition of SGLT2. Accordingly, a modest increase in drug levels would not necessarily be expected to increase the magnitude of glucosuria. It is also important to note that treatment emergent cases of ketoacidosis have been observed in SGLT2 inhibitors even in the absence of probenecid.

2. Role of glucuronidation in metabolic clearance of SGLTL2 inhibitors. Sarashina et al. (Drug Metab Pharmacokinet, 28, 213, 2013) reported that ~23% of the administered dose of empagliflozin was excreted in the urine. Macha et al. (Diabetes, Obesity and Metabolism, 16: 215, 2014) reported that mild-moderate renal impairment was associated with a modest increase (18-20%) in empagliflozin exposure while end-stage kidney disease was associated

with a 48% increase in empagliflozin exposure. Thus, renal mechanisms are not believed to be the principal route of elimination/degradation for SGLT2 inhibitors. Rather, glucuronidation in the liver is believed to be the principal mechanism whereby SGLT2 inhibitors are cleared from the circulation.

3. Delivery of drug to its site of action in the proximal tubule. Fu et al (Am J Physiol Renal, 315, F386, 2018) [reference #5 in the paper by Martin et al.] reported that genetic knockout of *Oat3* in mice decreased the effect of empagliflozin (sub-maximally effective doses) upon glucosuria. When administered at a dose of 30 mg/kg, empagliflozin induced similar degrees of glucosuria in both wild type and *Oat3* -/- mice. Based on these data in mice, one would predict that an inhibitor of OAT3 would likely not affect the pharmacodynamic response to maximally effective doses of empagliflozin while possibly decreasing the efficacy of sub-maximally effective doses. This seems to be inconsistent with what Martin et al. have hypothesized (i.e., that probenecid increases the efficacy of empagliflozin). Of course, one cannot be certain that data in the mouse are predictive of human pharmacology.

4. A possible pharmacodynamic drug-drug interaction. Notwithstanding concerns related to the hypothesized mechanisms mediating the hypothesized drug-drug interaction, Martin et al. have provided thought-provoking circumstantial evidence suggesting that probenecid may indeed have contributed to the pathogenesis of this patient's ketoacidosis. Accordingly, it is important to consider whether probenecid might have triggered other mechanisms that might have contributed to pathogenesis of ketoacidosis. Eraly et al. (J Biol Chem, 281: 5072, 2006) reported that knockout of the *Oat1* gene in mice decreased urinary excretion of 3-hydroxybutyrate by ~65% and also caused a dramatic increase in plasma 3-hydroxybutyrate levels. Because the ligand specificity of OAT1 and OAT3 are reported to be overlapping (Nigam et al., Physiol Rev, 95, 83, 2013), it is tempting to speculate that probenecid might also impair renal excretion of ketone bodies. (It is noteworthy that acetoacetate and 3-hydroxybutyrate are both organic anions and therefore reasonable candidates to be transported by organic anion transporters.) Furthermore, Cohen et al. (Am J Physiol, 184: 91, 1956) reported that phlorizin (the prototype SGLT inhibitor and also the chemical structure on which empagliflozin is based) promotes renal tubular reabsorption of acetoacetate. By analogy to phlorizin, it is plausible that empagliflozin might decrease urinary excretion of ketone bodies. We, therefore, hypothesize that probenecid and empagliflozin might synergize to increase plasma ketone body levels by decreasing renal clearance of those metabolities. Of course, empagliflozin-induced glucosuria indirectly triggers increased ketogenesis, which also contributes to the pathogenesis of SGLT2 inhibitor-induced ketoacidosis (Taylor et al, J. Clin Endocrinol Metab, 100, 2849, 2015).

References

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Is the background of the case's history and progression described in sufficient detail?
Yes

Are enough details provided of any physical examination and diagnostic tests, treatment given and outcomes?
Yes

Is sufficient discussion included of the importance of the findings and their relevance to future understanding of disease processes, diagnosis or treatment?
Yes

Is the case presented with sufficient detail to be useful for other practitioners?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: I am a physician-scientist with a special interest in endocrinology and diabetes -- especially with respect to drugs to treat diabetes and obesity. Based on my work at Bristol-Myers Squibb where I participated in R&D leading to approval of dapagliflozin, I have specialized expertise related to SGLT2 inhibitors.

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.

Author Response 20 Feb 2024

William Martin

Thank you for your thorough review of this case report and constructive feedback. We have responded to your very insightful comments by:

- Providing more detailed discussion of the pharmacokinetic drug-drug interaction between empagliflozin and probenecid, and whether or not increased systemic exposure to empagliflozin alone would be sufficient explain the severe episode of diabetic ketoacidosis observed; and
- Proposing an alternative or complementary explanation for the episode of diabetic ketoacidosis arising due to the empagliflozin-probenecid drug-drug interaction, wherein both drugs act to diminish renal elimination of ketone bodies.

These points are highlighted in two new paragraphs in the Discussion of the case report.

They are also highlighted in the Abstract of the revised case report. We believe that the case report now more comprehensively captures the potential mechanisms of diabetic ketoacidosis arising in the setting of empagliflozin and probenecid therapy, and recommendations are made for future studies in this field

Competing Interests: No competing interests were disclosed.