An Acad Bras Cienc (2022) 94(Suppl. 3): e20211287 DOI 10.1590/0001-3765202220211287
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

HEALTH SCIENCES

Toxicological assessment of SGLT2 inhibitors metabolites using *in silico* approach

JÉSSICA B. DE JESUS, RAISSA A. DA CONCEIÇÃO, THAYNÁ R. MACHADO, MARIA L.C. BARBOSA, THAISA F.S. DOMINGOS, LUCIO M. CABRAL, CARLOS R. RODRIGUES, BÁRBARA ABRAHIM-VIEIRA & ALESSANDRA M.T. DE SOUZA

Abstract: Sodium-glucose cotransporter 2 inhibitors (SGLT2i) are the latest class of drugs approved to treat type 2 DM (T2DM). Although adverse effects are often caused by a metabolite rather than the drug itself, only the safety assessment of disproportionate drug metabolites is usually performed, which is of particular concern for drugs of chronic use, such as SGLT2i. Bearing this in mind, *in silico* tools are efficient strategies to reveal the risk assessment of metabolites, being endorsed by many regulatory agencies. Thereby, the goal of this study was to apply *in silico* methods to provide the metabolites toxicity assessment of the SGLT2i. Toxicological assessment from SGLT2i metabolites retrieved from the literature was estimated using the structure and/or statistical-based alert implemented in DataWarrior and ADMET predictor™ softwares. The drugs and their metabolites displayed no mutagenic, tumorigenic or cardiotoxic risks. Still, M1-2 and M3-1 were recognized as potential hepatotoxic compounds and M1-2, M1-3, M3-1, M3-2, M3-3 and M4-3, were estimated to have very toxic LD_{50} values in rats. All SGLT2i and the metabolites M3-4, M4-1 and M4-2, were predicted to have reproductive toxicity. These results support the awareness that metabolites may be potential mediators of drug-induced toxicities of the therapeutic agents.

Key words: diabetes, *in silico* toxicology, metabolism, SGLT2 inhibitors, SGLT2i metabolites.

INTRODUCTION

Diabetes Mellitus (DM) is a chronic progressive metabolic disorder with an increasing prevalence worldwide. Type 2 DM (T2DM) is the most common, accounting for around 90% of diabetes cases. T2DM is a non-insulin dependent DM, caused by insulin decreased sensitivity of target tissues (WHO 2020). As a result of this, blood glucose concentration increases, promoting increased excretion of both sodium and glucose in the urine (Guyton & Hall 2006).

Sodium-glucose cotransporter-2 inhibitors (SGLT2i) are the latest therapeutic class for T2DM. SGLT2i reduces the renal tubular glucose reabsorption, producing a reduction in blood glucose without stimulating insulin release (Hsia et al. 2017). Currently, there are four SGLT2i approved by FDA: canagliflozin (1) (Invokana®), dapagliflozin (2) (Farxiga®), empagliflozin (3) (Jardiance®), and ertugliflozin (4) (Steglatro®) (Hsia et al. 2017) (Figure 1).

The adverse events of SGLT2i include symptomatic hypotension, hypoglycemia, urinary tract infections, and mycotic infections (Halimi & Vergès 2014). Furthermore, DM is associated with chronic liver disease increase associated with cirrhosis, non-alcoholic fatty liver disease, alcoholic cirrhosis, chronic hepatitis C (CHC), and hemochromatosis (Li et

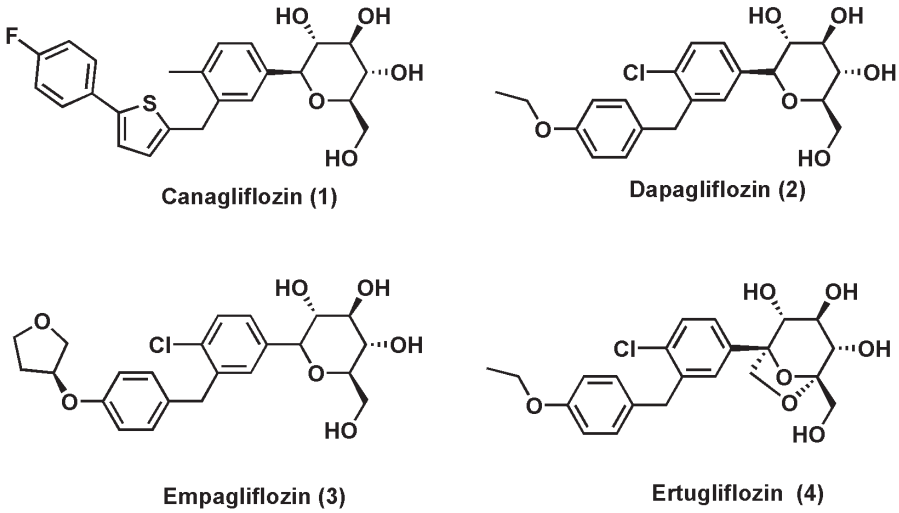

Figure 1. Chemical structures of SGLT2i: canagliflozin (1), dapagliflozin (2), empagliflozin (3) and ertugliflozin (4).

al. 2019, Kita et al. 2007). Previous reports also pointed out that T2DM is associated with an increased incidence of overall cancer (Tsilidis et al. 2015, Yuan et al. 2020). Phase III clinical trials with dapagliflozin (2) reported an imbalance of bladder cancer in men and breast cancer in women, which delayed its FDA approval (Burki 2012, Scheen 2014). An increased risk of bladder cancer was also observed in the individuals taking either empagliflozin (3) or dapagliflozin (2) (Tang et al. 2017, Scheen 2014). Corroborating these findings, a recent analysis reported a high number of cases of bladder cancer among users of SGLT2i (García et al. 2021). These adverse effects are a significant public health problem and a challenge for the pharmaceutical industry. Thus, further studies are required to support these pieces of evidence related to the SGLT2i therapy and long-term outcomes (Shao et al. 2020).

It is well-established that adverse effects often occur due to a metabolite rather than the drug itself (Park et al. 2001, Thompson et al. 2016, Mumtaz & Durkin 1992, Luffer-Atlas & Atrakchi 2017). Regulatory agencies recommend performing the safety assessment only for disproportionate drug metabolites, i.e., those present at > 10% of total drug-related human exposure at steady-state, while no tests are performed for the remaining metabolites. This lack of toxicological data is of particular concern since their contribution to the parent drug's overall toxicity remains unknown, particularly for metabolites of chronic use drugs (FDA 2020, Luffer-Atlas & Atrakchi 2017). Nowadays, computational methods play a vital role in the safety assessment of molecules with challenging isolation, quantification, or synthesis. *In silico* toxicology is one of the alternatives to animal testing to toxicity assessment that uses computational resources to organize, analyze, model, simulate, visualize, or predict the toxicity of chemicals (Raies & Bajic 2016). The use of computer-based models using machine learning and structural alert to predict toxicity has increased significantly due to improvements in the performance of the models and their ease of use (De Mello et al. 2018, Myatt et al. 2018, Graham et al. 2021). Also, *in silico* studies are being endorsed by regulatory agencies, as they are typically based on human data, with an enhancement of interspecies transferability. Mutagenicity, carcinogenicity, acute oral toxicity, liver adverse effects, allergenic skin sensitization, and reproductive toxicity are some of the toxicity endpoints usually estimated for chemicals (Archibald et al. 2018, Vedani & Smiesko 2009). In this context, *in silico* methods is an efficient

strategy to assess the safety profile of SGLT2i metabolites in humans.

MATERIALS AND METHODS

The metabolites of canagliflozin (1), dapagliflozin (2), empagliflozin (3), and ertugliflozin (4) generated in humans were retrieved from previous studies (Mamidi et al. 2014, Obermeier et al. 2010, Kasichayanula et al. 2014, Chen et al. 2015, Miao et al. 2013) and the two-dimensional structures were drawn with ACD/ChemSketch version 2020.2.0 (Advanced Chemistry Development, Inc., Toronto, ON, Canada).

The mutagenicity, hepatotoxicity, cardiotoxicity, reproductive toxicity and acute toxicity endpoints were evaluated using statistical-based models implemented in ADMET predictor™ version 9.5 (Simulations Plus, Inc., Lancaster, CA, USA, 2019).

The mutagenicity endpoint was predicted based on the Ames Test. This test applied models of Artificial Neural Network Ensembles as qualitative models for five strains of *Salmonella* (TA97 or TA1537, TA98, TA100, TA102, and TA1535 strains) with and without microsomal activation (Bakhtyari et al. 2013, Honma et al. 2019). Hepatotoxicity parameters were studied using five relevant biomarkers: alkaline phosphatase (ALP), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), gamma-glutamyl transferase (GGT), and lactate dehydrogenase (LDH) (Abreu et al. 2020, Garcia et al. 2021). The Cardiotoxicity model predicted the likelihood that a compound will block the hERG channel, related to ventricular arrhythmias and sudden death (Garcia et al. 2021). The reproductive toxicity endpoint includes anything that disturbs the reproductive process of organisms, including adverse effects to sexual organs, behavior, ease of conception, teratogenicity, and developmental toxicity to offspring before or after birth. Lastly, the acute toxicity model predicted the capability of the amount of orally administered chemical (mg/kg body weight) required to kill 50% of the rats population within 24 h of exposure (LD_{50}).

Besides statistical-based models, an expert rule-based method was also performed to evaluate mutagenicity and tumorigenicity using DataWarrior software (Sander et al. 2015, Guerra et al. 2017). The mutagenicity prediction includes 20 specific test systems, both *in vitro* and *in vivo,* with tested organisms including bacteria, molds, yeast, protozoa, insects, and mammalian cell lines. The tumorigenic effect data is predicted considering three criterias: carcinogenic by RTECS (Registry of Toxic Effects of Chemical Substances), neoplastic by RTECS, and equivocal tumorigenic results (Von Korff & Sander 2006, CDC 2011).

RESULTS

Compilation of SGLT2i metabolites

In vitro metabolic profiles from liver microsomes and hepatocyte incubations can be poor predictors of *in vivo* circulating major human metabolites (Luffer-Atlas & Atrakchi 2017). Thus, we retrieved from literature data of metabolic profile from SGLT2i after single oral dose administration to healthy humans (Mamidi et al. 2014, Obermeier et al. 2010, Kasichayanula et al. 2014, Chen et al. 2015, Miao et al. 2013). The biotransformation of SGLT2i in humans occurs primarily by *O*-glucuronidation and, to a lesser extent, by oxidation. Mamidi et al. (2014) identified three metabolites of canagliflozin (1): a hydroxylated canagliflozin by CYP3A4 (M1-1) and two pharmacologically inactive *O*-glucuronide conjugates by UGT2B4 (M1-2) and UGT1A9 (M1-3) (Figure 2) (Mamidi et al. 2014).

Two major metabolites of dapagliflozin (2) were identified in clinical samples, the

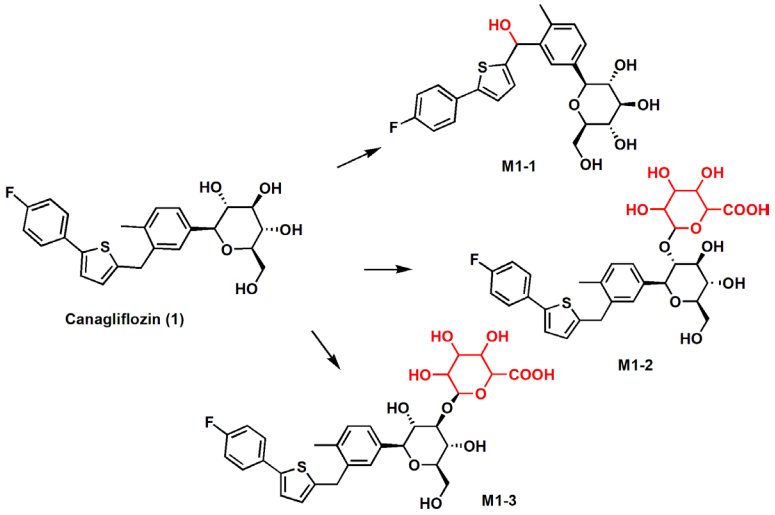

Figure 2. The chemical structures of metabolites M1-1, M1-2 and M1-3 of canagliflozin (1).

nonpharmacological active dapagliflozin 3-*O*-glucuronide (M2-1) and dapagliflozin 2-*O*-glucuronide (M2-2) (Figure 3) (Obermeier et al. 2010, Kasichayanula et al. 2014).

The *in vivo* biotransformation of empagliflozin (3) resulted in six metabolites described by Chen et al. (2015), but one of them is related as an oxidation/dehydrogenation metabolite and did not have its structure completely elucidated. Thereby, two metabolites were products from Phase I metabolism (M3-1 and M3-2) and three of them from glucuronidation by UGT1A9 (M3-3, M3-4, and M3-5) (Figure 4).

Oxidation of ertugliflozin (4) by CYP3A4 and 3A5 occurs to a minor extent to yield monohydroxylated metabolites (M4-1 and M4-2) whilst the main biotransformation pathway involves glucuronidation by UGT1A9 and 2B7, yielding two regioisomers metabolites (M4-3 and M4-4) (Figure 5) (Miao et al. 2013).

In silico toxicity assessment of metabolites

The *in silico* toxicity assessment of SGLT2i and its metabolites were performed using two methodologies, that complement each other, a statistical-based and an expert rule-based, from ADMET Predictor™ (Simulations Plus 2019) and DataWarrior, respectively (Sander et al. 2015, Guerra et al. 2017) (Table I). Statistical-based models apply machine learning algorithms to analyze the correlations between molecular structures and biological activity. Expert rule-based models find the compounds most similar to the parent compound based on similarity while leaving the selected structure untouched using fragments from known drugs (Goel & Valerio Jr 2020, Sander et al. 2015).

The mutagenicity predictions indicated that neither the drugs nor their metabolites might be mutagenic, considering results from the statistical-based model (Table I). In the expert rule-based analysis, ertugliflozin (4) and its metabolites presented mutagenic potential (Table I). Also, DataWarrior results indicated that all SGLT2i and metabolites are not tumorigenic based on RTECS criteria (Table I).

Cardiotoxicity evaluation indicated that these metabolites do not interact with the hERG potassium channel (Table II). Concerning hepatotoxicity, only canagliflozin and empagliflozin metabolites M1-2 and M3-1, respectively, presented potential risk (Table II). All SGLT2i, the empagliflozin metabolite M3-4, and the ertugliflozin metabolites M4-1 and M4-2 presented reproductive toxicity risk. Finally, solely the *O*-glucuronide metabolites of canagliflozin M1-2 and M1-3 presented acute rat toxicity (Table II).

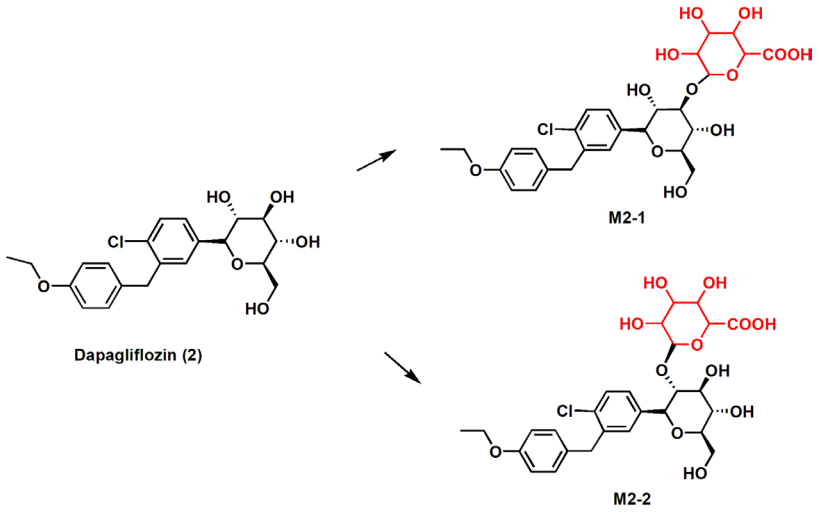

Figure 3. The chemical structures of the metabolites for dapagliflozin (2).

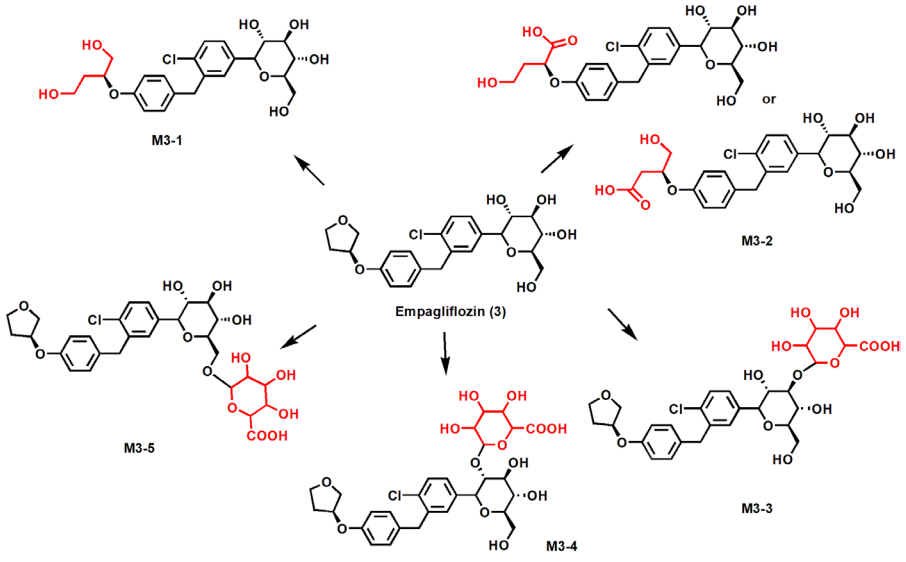

Figure 4. The chemical structures of empagliflozin (3) and its metabolites.

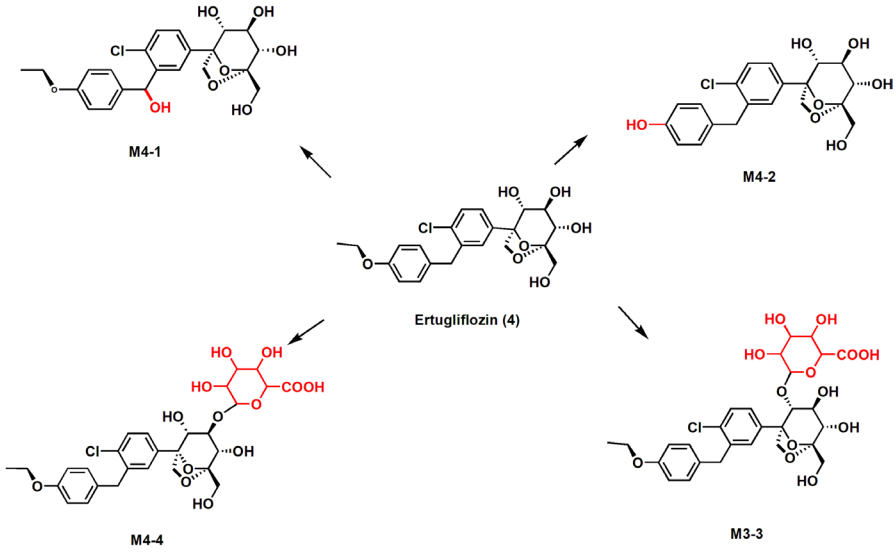

Figure 5. The chemical structures of the metabolites for ertugliflozin (4).

Table I. Mutagenic and tumorigenic prediction of SGLT2i and its metabolites.

Drug/ Metabolites	ADMET Predictor	DataWarrior	
	Mutagenic	Mutagenic	Tumorigenic
Canagliflozin	No	No	No
M1-1	No	No	No
M1-2	No	No	No
M1-3	No	No	No
Dapagliflozin	No	No	No
M2-1	No	No	No
M2-2	No	No	No
Empagliflozin	No	No	No
M3-1	No	No	No
M3-2	No	No	No
M3-3	No	No	No
M3-4	No	No	No
M3-5	No	No	No
Ertugliflozin	No	Yes	No
M4-1	No	Yes	No
M4-2	No	Yes	No
M4-3	No	Yes	No
M4-4	No	Yes	No

DISCUSSION

Nowadays, the increasing interest and acceptance of *in silico* methods are providing their inclusion for regulatory purposes. Based on the FDA recommended studies for assessing the safety of the disproportionate metabolites (FDA 2020), we conducted mutagenicity, tumorigenicity, and general toxicity studies for the SGLT2i metabolites using established *in silico* approaches (National Research Council 2007). Mutagenicity evaluations were carried out using two different approaches: statistical-based and expert-system rule-based (ICH 2015). The statistical-based model presented non-mutagenicity results for all drugs and their metabolites corroborating the data from drug registration (Table I). In the expert rule-based analysis, ertugliflozin (4) and its metabolites (M4-1 to M4-4) presented structural alert, which suggest the potential mutagenicity of the dioxabicyclo[3.2.1]octane moiety. Indeed, non-mutagenic data was attributed to this group in the literature and the statistical-based method does not indicate this toxicity. In order to rationalize the final conclusion, the expert knowledge was applied to support this evidence, deciding that non-mutagenic results were linked to these metabolites (ICH 2015).

Carcinogenicity studies should be conducted on metabolites of drugs that are used regularly for at least 6 months or for the treatment of chronic diseases. Hence, we also conducted carcinogenicity evaluation of SGLT2i

Table II. ***In silico*** **cardiotoxicity, hepatotoxicity and acute toxicity in rats (LD_{50}) of SGLT2i and its metabolites using ADMET Predictor™.**

Drug/ Metabolites	ADMET Predictor	DataWarrior	
	Mutagenic	Mutagenic	Tumorigenic
Canagliflozin	No	No	No
M1-1	No	No	No
M1-2	No	No	No
M1-3	No	No	No
Dapagliflozin	No	No	No
M2-1	No	No	No
M2-2	No	No	No
Empagliflozin	No	No	No
M3-1	No	No	No
M3-2	No	No	No
M3-3	No	No	No
M3-4	No	No	No
M3-5	No	No	No
Ertugliflozin	No	Yes	No
M4-1	No	Yes	No
M4-2	No	Yes	No
M4-3	No	Yes	No
M4-4	No	Yes	No

Drug/ Metabolites	hERG inhibitor	Hepatotoxicity	Acute Toxicity in rats (LD_{50}, mg/kg)	Reproductive toxicity
Canagliflozin	No	No	621	Yes
M1-1	No	No	586	No
M1-2	No	Yes	153	No
M1-3	No	No	208	No
Dapagliflozin	No	No	723	Yes
M2-1	No	No	778	No
M2-2	No	No	783	No
Empagliflozin	No	No	435	Yes
M3-1	No	Yes	370	No
M3-2	No	No	428	No

Table II. Contnuation.

M3-3	No	No	385	No
M3-4	No	No	754	Yes
M3-5	No	No	848	No
Ertugliflozin	No	No	510	Yes
M4-1	No	No	520	Yes
M4-2	No	No	612	Yes
M4-3	No	No	495	No
M4-4	No	No	523	No

metabolites (FDA 2020). DataWarrior results indicated low tumorigenic risk to all SGLT2i and its metabolites, following SGLT2i clinical outcomes (Janssen Pharmaceutical Companies 2013, Boehringer Ingelheim Pharmaceuticals 2014, Bristol-Myers Squibb Company 2014, Merck Sharp & Dohme Corp. 2017, Tang et al. 2017). For this endpoint, SGLT2i and its metabolites were outside the applicability domain (AD) of the carcinogenicity model implemented in ADMET Predictor™. In other words, these molecules were out-of-scope and the predictions were not considered due to its low reliability (Simulations Plus 2019, Ruiz et al. 2017, El-Saadi et al. 2015).

None of the SGLT2i metabolites were predicted to interact with the hERG potassium channel. These data may be supported by the clinical evidence for cardioprotective effects of SGLT2i (Table II) (Rahman et al. 2017, Simulations Plus 2019).

Evidence for the cardiovascular benefits of SGLT2i continues to accumulate. Treatment with SGLT2 inhibitors has been linked to a reduced risk of heart failure and cardiovascular death in clinical trials (Sayour et al. 2021).

While some parent drugs can directly cause hepatotoxicity, it is generally the metabolites of these compounds that lead to liver injury (Tarantino et al. 2009). Thus, it is crucial to evaluate the hepatotoxicity of SGTL2i metabolites. Due to SGOT and SGPT increased levels, canagliflozin and empagliflozin metabolites (M1-2 and M3-1, respectively) are potential hepatotoxic compounds (Table II). Indeed, in multiple large randomized controlled trials, the hepatotoxicity of SGLT2i was unproven but suspected to be a rare cause of clinically apparent liver injury due to serum enzyme elevations (Livertox 2012). Furthermore, it has been suggested that for every 10 SGPT cases reported in a clinical trial, there will be one case of more severe liver injury. This case develops once the drug is of chronic use (Bell & Chalasani 2009). Since the canagliflozin (1) and empagliflozin (3) metabolites (M1-2 and M3-1, respectively) cause hepatotoxicity, it may affect the metabolism of other drugs (Bell & Chalasani 2009, Tarantino et al. 2009). In addition to M1-2 hepatotoxicity, the starting dose of canagliflozin (1) is 100 mg/day orally (Janssen Pharmaceutical Companies 2013). Therefore, these outcomes lead to a possible safety concern, as daily doses of ≥ 50 mg are significantly more likely to cause liver injury (Lammert et al. 2008). Furthermore, SGLT2i shares similar pharmacokinetic characteristics, with an extensive hepatic metabolism, mainly via glucuronidation (Scheen 2015). And there is a higher number of liver injury cases from drugs that undergo significant hepatic metabolism (Chan & Benet 2017). On the other hand, the recommended empagliflozin (3) starting dose is 10 mg once daily (Boehringer

Ingelheim Pharmaceuticals 2014), which leads to a less relevant safety concern compared with canagliflozin metabolite.

The acute toxicity endpoint predicts the amount of orally administered substance (in mg/kg body weight) required to kill 50% of the rats tested (LD_{50}) (Simulations Plus 2019). The rat oral LD_{50} ADMET Predictor™ model is supported by data from two sources, CDC's Registry of Toxic Effects of Chemical Substances (RTECS), and the ChemIDplus database (Ruiz et al. 2012). One of the most common scales used for the final interpretation of acute rat toxicity results is the Hodge and Sterner scale (Hodge & Sterner 2005, Erhirhie et al. 2018, Ruiz et al. 2012). According to toxicity classes of Hodge & Sterner (2005), the *O*-glucuronide metabolites of canagliflozin M1-2 and M1-3, empagliflozin (3) and its metabolites M3-1, M3-2 and M3-3, and ertugliflozin metabolite M4-3 were estimated to have worrying toxicity, with LD_{50} values <500mg/kg. The remaining SGLT2i and its metabolites were estimated to have moderately toxic level, with LD_{50} values (500–5000 mg/kg) (Table II). The Globally Harmonized System (GHS) of Classification and Labelling of Chemicals criteria are used to determine the nature and the relative severity of the hazard of a chemical substance or mixture. According to GHS criteria, chemicals are assigned to one of the five toxicity categories on the basis of LD_{50} (oral, dermal) or LC_{50} (inhalation) from acute toxicity studies (United Nations 2021). The LD_{50} of the *O*-glucuronide metabolites of canagliflozin M1-2 and M1-3 places them within the category 3 of the Organization for Economic Cooperation and Development (OECD) GHS classification system (LD_{50}<300 mg/kg), while the SGLT2i and the remaining metabolites were classified in the less severe hazard category 4 (300 < LD_{50} < 2000 mg/kg). Furthermore, the European Union regulation is currently furthering alternative toxicity studies that decrease the use of animals. Consequently, this impacts specific regulations, for instance, REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) (Diaza et al. 2015).

All SGLT2i, the empagliflozin metabolite M3-4, and the ertugliflozin metabolites M4-1 and M4-2 presented reproductive toxicity risk (Table II). Clinical trial evidence (Monami et al. 2014, Rizzi & Trevisan 2016, Zaccardi et al. 2016) and post-marketing safety analysis corroborates these findings (Raschi et al. 2017). According to this analysis, SGLT2i are associated with a high report of reproductive adverse effects in the international pharmacovigilance databases. Signals of reproductive events in the post-marketing analysis were largely in agreement with data obtained from pre-approval RCTs (Randomized control trials) (Monami et al. 2014, Rizzi & Trevisan 2016, Zaccardi et al. 2016). In addition to this clinical evidence, these results reinforced the guidance that prescribers should be aware of these common safety issues and should monitor patients to avoid them. These results support the awareness that metabolites may be potential mediators of drug-induced toxicities of the therapeutic agents and should be structurally and toxicologically characterized.

CONCLUSIONS

It is well-established that metabolite rather than the drug itself causes adverse reactions. Herein, we showed that all SGLT2i and its metabolites were non-tumorigenic, non-mutagenic and non-cardiotoxic. However, particular attention must be given to canagliflozin and empagliflozin metabolites M1-2, M1-3, M3-1, M3-2 and M3-3, which present hepatotoxicity (M1-2 and M3-1) and high LD_{50} values according to Hodge and Sterner scale (M1-2, M1-3, M3-1, M3-2 and M3-3). In agreement with clinical trials evidence and post-marketing analysis, all SGLT2i, the

empagliflozin M3-4, and ertugliflozin M4-1 and M4-2 were predicted to have reproductive toxicity. Concerning these endpoints, our *in silico* results support the awareness that even minority metabolites may be potential mediators of drug-induced toxicities, especially for drugs of chronic use, and thus should be evaluated by sponsors and regulators.

Acknowledgments

This study was partially financed by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil/CAPES - Finance Code 001, by Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro - Brazil/FAPERJ, Institutos Nacionais de Ciência e Tecnologia-Conselho Nacional de Desenvolvimento Científico e Tecnológico - Brazil/CNPq.

REFERENCES

ABREU LCL, ABRAHIM-VIEIRA BA, SOUZA AMT, PINTO EC, GONÇALVES MS, SIMON A, VIANA GM, RODRIGUES CR, SOUSA VP & CABRAL LM. 2020. Forced degradation studies of norepinephrine and epinephrine from dental anesthetics: development of stability-indicating HPLC method and in silico toxicity evaluation. Biomed Chromatogr 34: e4832.

ARCHIBALD K, TSAIOUN K, KENNA JG & POUND P. 2018. Better science for safer medicines: the human imperative. J R Soc Med 111: 433-438.

BAKHTYARI NG, RAITANO G, BENFENATI E, MARTIN T & YOUNG D. 2013. Comparison of in silico models for prediction of mutagenicity. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev 31: 45-66.

BELL LN & CHALASANI N. 2009. Epidemiology of idiosyncratic drug-induced liver injury. Semin Liver Dis 29: 337-347.

BOEHRINGER INGELHEIM PHARMACEUTICALS. 2014. Jardiance (empagliflozin) [package insert], Ridgefield, CT: Boehringer Ingelheim, p. 1-39.

BRISTOL-MYERS SQUIBB COMPANY. 2014. Farxiga (dapagliflozin) [package insert]. Princeton, NJ: Bristol-Myers Squibb Company, p. 1-43.

BURKI TK. 2012. FDA rejects novel diabetes drug over safety fears. Lancet 379: 507.

CDC – CENTERS FOR DISEASE CONTROL AND PREVENTION. 2011. Features of the RTECS Database (June 2011). Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. https://www.cdc.gov/niosh/rtecs/rtecsfeatures.html. Accessed 1 April 2021.

CHAN R & BENET LZ. 2017. Evaluation of DILI Predictive Hypotheses in Early Drug Development. Chem Res Toxicol 30: 1017-1029.

CHEN LZ, JUNGNIK A, MAO Y, PHILIP E, SHARP D, UNSELD A, SEMAN L, WOERLE HJ & MACHA S. 2015. Biotransformation and mass balance of the SGLT2 inhibitor empagliflozin in healthy volunteers. Xenobiotica 45: 520-529.

DE MELLO MVP, ABRAHIM-VIEIRA BDA, DOMINGOS TFS, DE JESUS JB, SOUSA ACC, RODRIGUES CR & SOUZA AMT. 2018. A comprehensive review of chalcone derivatives as antileishmanial agents. Eur J Med Chem 150: 920-929.

DIAZA RG, MANGANELLI S, ESPOSITO A, RONCAGLIONI A, MANGANARO A & BENFENATI E. 2015. Comparison of in silico tools for evaluating rat oral acute toxicity. SAR QSAR Environ Res 26: 1-27.

EL-SAADI MW, WILLIAMS-HART T, SALVATORE BA & MAHDAVIAN E. 2015. Use of in-silico assays to characterize the ADMET profile and identify potential therapeutic targets of fusarochromanone, a novel anti-cancer agent. In Silico Pharmacol 3: 6.

ERHIRHIE EO, IHEKWEREME CP & ILODIGWE EE. 2018. Advances in acute toxicity testing: strengths, weaknesses and regulatory acceptance. Interdiscip Toxicol 11: 5-12.

FDA – US FOOD AND DRUG ADMINISTRATION. 2020. Center for Drug Evaluation and Research (CDER). Safety Testing of Drug Metabolites - Guidance for Industry, p. 1-11.

GARCIA AR ET AL. 2021. Identification of Chalcone Derivatives as Inhibitors of *Leishmania infantum* Arginase and Promising Antileishmanial Agents. Front Chem 8: 624678: 1-10.

GARCÍA M, ARTECHE-MARTINEZ U, LERTXUNDI U & AGUIRRE C. 2021. SGLT2 Inhibitors and Bladder Cancer: Analysis of Cases Reported in the European Pharmacovigilance Database. J Clin Pharmacol 61: 187-192.

GOEL R & VALERIO JR LG. 2020. Predicting the mutagenic potential of chemicals in tobacco products using in silico toxicology tools. Toxicol Mech Methods 30: 672-678.

GRAHAM JC, RODAS M, HILLEGASS J & SCHULZE G. 2021. The performance, reliability and potential application of in silico models for predicting the acute oral toxicity of pharmaceutical compounds. Regul Toxicol Pharmacol 119: 104816.

GUERRA LR, DE SOUZA AMT, CÔRTES JA, LIONE V, CASTRO HC & ALVES GG. 2017. Assessment of predictivity of volatile

organic compounds carcinogenicity and mutagenicity by freeware in silico models. Regul Toxicol Pharmacol 91: 1-8.

GUYTON AC & HALL JE. 2006. Textbook of Medical physiology. 11th ed, Amsterdam: Elsevier Inc, 1168 p.

HALIMI S & VERGÈS B. 2014. Adverse effects and safety of SGLT-2 inhibitors. Diabetes Metab 40: S28-S34.

HODGE A & STERNER B. 2005. Toxicity Classes. In: Canadian Center for Occupational Health and Safety. https://www.ccohs.ca/oshanswers/chemicals/ld50.html. Accessed 20 October 2021.

HONMA M ET AL. 2019. Improvement of quantitative structure-activity relationship (QSAR) tools for predicting Ames mutagenicity: outcomes of the Ames/QSAR International Challenge Project. Mutagenesis 34: 3-16.

HSIA DS, GROVE O & CEFALU WT. 2017. An update on sodium-glucose co-transporter-2 inhibitors for the treatment of diabetes mellitus. Curr Opin Endocrinol Diabetes Obes 24: 73-79.

ICH. 2015. EMA/CHMP/ICH/83812/2013: ICH guideline M7(R1) on assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk, p. 1-110.

JANSSEN PHARMACEUTICAL COMPANIES. 2013. Invokana (canagliflozin) [package insert]. Titusville, NJ: Janssen Pharmaceutical, p. 1-19.

KASICHAYANULA S, LIU X, LACRETA F, GRIFFEN SC & BOULTON DW. 2014. Clinical pharmacokinetics and pharmacodynamics of dapagliflozin, a selective inhibitor of sodium-glucose co-transporter type 2. Clin. Pharmacokinet 53: 17-27.

KITA Y, MIZUKOSHI E, TAKAMURA T, SAKURAI M, TAKATA Y, ARAI K, YAMASHITA T, NAKAMOTO Y & KANEKO S. 2007. Impact of diabetes mellitus on prognosis of patients infected with hepatitis C virus. Metabolism 56: 1682-1688.

LAMMERT C, EINARSSON S, SAHA C, NIKLASSON A, BJORNSSON E & CHALASANI N. 2008. Relationship between daily dose of oral medications and idiosyncratic drug-induced liver injury: search for signals. Hepatology 47: 2003-2009.

LI X, JIAO Y, XING Y & GAO P. 2019. Diabetes Mellitus and Risk of Hepatic Fibrosis/Cirrhosis. Biomed Res Int 2019: 5308308.

LIVERTOX. 2012. LiverTox: Clinical and Research Information on Drug-Induced Liver Injury [Internet]. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases. SGLT-2 Inhibitors. [Updated 2018 Dec 17]. Available at: https://www.ncbi.nlm.nih.gov/books/NBK548289, p. 1-10.

LUFFER-ATLAS D & ATRAKCHI A. 2017. A decade of drug metabolite safety testing: industry and regulatory shared learning. Expert Opin Drug Metab Toxicol 13: 897-900.

MAMIDI RN ET AL. 2014. Metabolism and excretion of canagliflozin in mice, rats, dogs, and humans. Drug Metab Dispos 42: 903-916.

MERCK SHARP & DOHME CORP. 2017. Steglatro (ertugliflozin) [package insert]. Whitehouse Station, NJ: Merck Sharp & Dohme Corp, p. 1-23.

MIAO Z, NUCCI G, AMIN N, SHARMA R, MASCITTI V, TUGNAIT M, VAZ AD, CALLEGARI E & KALGUTKAR AS. 2013. Pharmacokinetics, metabolism, and excretion of the antidiabetic agent ertugliflozin (PF-04971729) in healthy male subjects. Drug Metab Dispos 41: 445-456.

MONAMI M, NARDINI C & MANNUCCI E. 2014. Efficacy and safety of sodium glucose co-transport-2 inhibitors in type 2 diabetes: a meta-analysis of randomized clinical trials. Diabetes Obes Metab 16: 457-466.

MUMTAZ MM & DURKIN PR. 1992. A weight-of-evidence approach for assessing interactions in chemical mixtures. Toxicol Ind Health 8: 377-406.

MYATT GJ ET AL. 2018. In silico toxicology protocols. Regul Toxicol Pharmacol 96: 1-17.

NATIONAL RESEARCH COUNCIL. 2007. Toxicity Testing in the 21st Century: A Vision and a Strategy. Washington: The National Academies Press, 216 p.

OBERMEIER M ET AL. 2010. In vitro characterization and pharmacokinetics of dapagliflozin (BMS-512148), a potent sodium-glucose cotransporter type II inhibitor, in animals and humans. Drug Metab Dispos 38: 405-414.

PARK BK, KITTERINGHAM NR, KENNY JR & PIRMOHAMED M. 2001. Drug metabolism and drug toxicity. Inflammopharmacology 9: 183-199.

RAHMAN A, HITOMI H & NISHIYAMA A. 2017. Cardioprotective effects of SGLT2 inhibitors are possibly associated with normalization of the circadian rhythm of blood pressure. Hypertension Res 40: 535-540.

RAIES AB & BAJIC VB. 2016. In silico toxicology: computational methods for the prediction of chemical toxicity. Wiley Interdiscip Rev Comput Mol Sci 6: 147-172.

RASCHI E, PARISOTTO M, FORCESI E, LA PLACA M, MARCHESINI G, DE PONTI F & POLUZZI E. 2017. Adverse events with sodium-glucose co-transporter-2 inhibitors: a global analysis of international spontaneous reporting systems. Nutr Metab Cardiovasc Dis 12: 1098-1107.

RIZZI M & TREVISAN R. 2016. Genitourinary infections in diabetic patients in the new era of diabetes therapy with sodium glucose cotransporter-2 inhibitors. Nutr Metab Cardiovasc Dis 26: 963-970.

RUIZ P, BEGLUITTI G, TINCHER T, WHEELER J & MUMTAZ M. 2012. Prediction of acute mammalian toxicity using QSAR methods: a case study of sulfur mustard and its breakdown products. Molecules (Basel, Switzerland) 17: 8982-9001.

RUIZ P, SACK A, WAMPOLE M, BOBST S & VRACKO M. 2017. Integration of *in silico* methods and computational systems biology to explore endocrine-disrupting chemical binding with nuclear hormone receptors. Chemosphere 178: 99-109.

SANDER T, FREYSS J, VON KORFF M & RUFENER C. 2015. DataWarrior: an open-source program for chemistry aware data visualization and analysis. J Chem Inf Model 55: 460-473.

SAYOUR AA, CELENG C, OLÁH A, RUPPERT M, MERKELY B & RADOVITS T. 2021. Sodium–glucose cotransporter 2 inhibitors reduce myocardial infarct size in preclinical animal models of myocardial ischaemia–reperfusion injury: a meta-analysis. Diabetologia 64: 737-748.

SCHEEN AJ. 2014. Evaluating SGLT2 inhibitors for type 2 diabetes: pharmacokinetic and toxicological considerations. Expert Opin Drug Metab Toxicol 10: 647-663.

SCHEEN AJ. 2015. Pharmacokinetics, Pharmacodynamics and Clinical Use of SGLT2 Inhibitors in Patients with Type 2 Diabetes Mellitus and Chronic Kidney Disease. Clin Pharmacokinet 54: 691-708.

SHAO SC, KUO LT, CHIEN RN, HUNG MJ & LAI EC. 2020. SGLT2 inhibitors in patients with type 2 diabetes with non-alcoholic fatty liver diseases: an umbrella review of systematic reviews. BMJ Open Diabetes Res Care 8: e001956.

SIMULATIONS PLUS. 2019. Simulations Plus, Inc., Lancaster, CA, USA.

TANG H, DAI Q, SHI W, ZHAI S, SONG Y & HAN J. 2017. SGLT2 inhibitors and risk of cancer in type 2 diabetes: a systematic review and meta-analysis of randomised controlled trials. Diabetologia 60: 1862-1872.

TARANTINO G, DI MINNO MND & CAPONE D. 2009. Drug-induced liver injury: Is it somehow foreseeable? World J Gastroenterol 15: 2817-2833.

THOMPSON RA, ISIN EM, OGESE MO, METTETAL JT & WILLIAMS DP. 2016. Reactive Metabolites: Current and Emerging Risk and Hazard Assessments. Chem Res Toxicol 29: 505-533.

TSILIDIS KK, KASIMIS JC, LOPEZ DS, NTZANI EE & IOANNIDIS JP. 2015. Type 2 diabetes and cancer: umbrella review of meta-analyses of observational studies. BMJ 350: g7607.

UNITED NATIONS. 2021. Globally harmonized system of classification and labelling of chemicals (GHS). Ninth revised edition. New York: United Nations, 556 p.

VEDANI A & SMIESKO M. 2009. In silico toxicology in drug discovery - concepts based on three-dimensional models. ATLA 37: 477-496.

VON KORFF M & SANDER T. 2006. Toxicity-indicating structural patterns. J Chem Inf Model 46: 536-544.

WHO – WORLD HEALTH ORGANIZATION. 2020. Diabetes. https://www.who.int/news-room/fact-sheets/detail/diabetes. Accessed 1 April 2021.

YUAN S, KAR S, CARTER P, VITHAYATHIL M, MASON AM, BURGESS S & LARSSON SC. 2020. Is Type 2 Diabetes Causally Associated With Cancer Risk? Evidence From a Two-Sample Mendelian Randomization Study. Diabetes 69: 1588-1596.

ZACCARDI F, WEBB DR, HTIKE ZZ, YOUSSEF D, KHUNTI K & DAVIES MJ. 2016. Efficacy and safety of sodium-glucose co-transporter-2 inhibitors in type 2 diabetes mellitus: systematic review and network meta-analysis. Diabetes Obes Metab 18: 783-794.

How to cite

DE JESUS JB, DA CONCEIÇÃO RA, MACHADO TR, BARBOSA MLC, DOMINGOS TFS, CABRAL LM, RODRIGUES CR, ABRAHIM-VIEIRA B & DE SOUZA AMT. 2022. Toxicological assessment of SGLT2 inhibitors metabolites using *in silico* approach. An Acad Bras Cienc 94: e20211287. DOI 10.1590/0001-3765202220211287.

Manuscript received on September 20, 2021;
accepted for publication on February 1, 2022

JÉSSICA B. DE JESUS[1]
https://orcid.org/0000-0003-0105-5815

RAISSA A. DA CONCEIÇÃO[1]
https://orcid.org/0000-0001-8264-7673

THAYNÁ R. MACHADO[1]
https://orcid.org/0000-0001-8020-6938

MARIA L.C. BARBOSA[1]
https://orcid.org/0000-0002-1764-8650

THAISA F.S. DOMINGOS[2]
https://orcid.org/0000-0002-7960-2543

LUCIO M. CABRAL[1]

https://orcid.org/0000-0002-4550-5729

CARLOS R. RODRIGUES[1]
https://orcid.org/0000-0001-8453-7654

BÁRBARA ABRAHIM-VIEIRA[1]
https://orcid.org/0000-0001-6444-0169

ALESSANDRA M.T. DE SOUZA[1]
https://orcid.org/0000-0001-9986-405X

[1]Universidade Federal do Rio de Janeiro, Faculdade de Farmácia, Departamento de Fármacos e Medicamentos, Av. Carlos Chagas Filho, 373, CCS, Bloco Lss, Cidade Universitária, 21941-902 Rio de Janeiro, RJ, Brazil

[2]BIODATA Computing Services & Consulting, Rua Aloísio Teixeira, 278, Parque Tecnológico, Cidade Universitária, 21941-850 Rio de Janeiro, RJ, Brazil

Correspondence to: **Bárbara Abrahim-Vieira, Alessandra M.T. de Souza**
E-mail: barbaraabrahim@pharma.ufrj.br, amtsouza@pharma.ufrj.br

Author contributions

A.M.T.S and B.A.V. conceived the initial idea, designed and directed the research. J.B.J., R.AC., T.R.M and T.F.S.D. performed the bibliographic search and toxicity analysis of all metabolites. J.B.J., R.A.C. and T.R.M wrote the draft manuscript. C.R.R. and L.M.C. supported all the computational work. A.M.T.S., B.A.V. and M.L.C.B. finalized the manuscript and all authors approved it.