SHORT COMMUNICATION

Apigenin and kaempferol as novel renoprotective agent against cisplatin-induced toxicity: an in vitro study

Abhilasha Sharmaa,b#, Sonam Sinhaa,c# and Neeta Shrivastavaa,d

aB. V. Patel Pharmaceutical Education and Research Development (PERD) Centre, Ahmedabad, Gujarat, India; bDepartment of Life science, Gujarat University, Ahmedabad, Gujarat, India; cKashiv Biosciences, Ahmedabad, Gujarat, India; dShri B.V. Patel Education Trust, Ahmedabad, Gujarat, India

ABSTRACT

Cisplatin is one of the highly consumed and potent antineoplastic drugs. However, its side effects in normal tissues, notably nephrotoxicity, is a major stumbling block and dose-limiting factor. Renoprotective approaches are being developed, however, the protective benefits are usually only partial implying the need for combinatorial strategies. Therefore, in this study, we investigated the nephroprotective efficacy of apigenin and kaempferol as dietary supplements against cisplatin-induced renal injury using human embryonic kidney (HEK-293) cells as our in vitro model. Our findings from MTT data, morphology studies, comet and ROS analysis suggest that CIS 11.36 μM + API 12.5 μg/mL and CIS 11.36 μM + KMP 25 μg/mL protects against cisplatin-induced nephrotoxicity. Results of western blot analysis further suggest the involvement of NGAL in the API and KMP mediated nephroprotection. Collectively, our studies suggest that API and KMP are promising candidates to be further developed as renoprotective agents against cisplatin-induced toxicity.

ARTICLE HISTORY

Received 22 October 2021
Accepted 19 February 2022

KEYWORDS

Cisplatin; apigenin; kaempferol; nephrotoxicity; renal protection; chemotherapy

CONTACT Neeta Shrivastava neetashrivastava@hotmail.com

Supplemental data for this article can be accessed online at https://doi.org/10.1080/14786419.2022.2045603.

#Authors have an equal contribution.

© 2022 Informa UK Limited, trading as Taylor & Francis Group
1. Introduction

Cisplatin is a potent antineoplastic drug that is being clinically used to treat various human malignancies including lung cancer, stomach cancer, breast cancer etc. (Dasari and Tchounwou 2014). Unlike other chemotherapeutic drugs, which are generally, complex organic components, cisplatin is a simple inorganic chemical. Cisplatin’s antineoplastic actions are mediated by a variety of cytotoxic mechanisms. It is believed that cisplatin binds to DNA, leading to the formation of inter-and intrastrand cross-links. The cross-linking results in defects in DNA templates, cross-arrest of DNA synthesis and replication and hence induces DNA damage. It is also known for activating apoptotic pathways and inflicts cellular damage via oxidative stress and inflammation (Zamble and Lippard 1995; Siddik 2003; Florea and Büsselberg 2011).

Despite the fact that cisplatin has been a mainstay chemotherapeutic drug, its antineoplastic activity causes persistent damage to normal tissues. Also, cisplatin is excreted by the kidneys and can build up in the proximal tubules, causing nephrotoxicity. However, its nephrotoxic effect is a chief impediment and a dose-limiting factor for anti-cancer therapy. Clinically, individuals receiving cisplatin have a 20%—30% chance of developing nephrotoxicity, which can result in mortality in acute kidney injury patients (Gonzalez-Vitale et al. 1977; Pierson-Marchandise et al. 2017). Decreased renal tubular function, abrupt renal failure, a decrease in whole blood cells, anaemia, muscular tremors, weight loss, gastrointestinal dysfunction, lethargy and orbital tightness are all symptoms of cancer patients with acute kidney injury, all of which limit the use of cisplatin for anticancer treatment (Effects and side-effects of cisplatin, 1982). Although the mechanisms underlying cisplatin-induced nephrotoxicity is not entirely comprehended, however, much like its anti-neoplastic effects, DNA damage, apoptotic, oxidative stress and inflammation are reported to be involved in the advancement and augmentation of kidney injury (Filipski et al. 2009; Pabla et al. 2009).
Currently, there is no clinically medication that can prevent or cure cisplatin-induced nephrotoxicity in humans. Dietary therapeutics of the nutrition field has become one of the most widespread trends as dietary supplements are not only beneficial in so many ways but are harmless also. Several bodies of evidence have underlined that many medicinal dietary supplements have the potential to become valuable complementary therapy for various renal disorders. Reports also suggest that naturally occurring dietary substances possess antioxidant properties and could be used for the mitigation of drug-induced nephrotoxicity. Further, many medicinal plants have been asserted to have protecting effect against renal injury and urinary ailments in folklore and traditional medicine.

In the current study, Apigenin (API) and Kaempferol (KMP) were selected as dietary supplements to investigate its protective effect against cisplatin-induced nephrotoxicity. API and KMP were selected owing to their plethora of biological activities that have been reported in different studies displaying their therapeutic and protective effects. API and KMP have been reported for their anti-inflammatory, anti-oxidant, anti-genotoxic and anti-cancerous effects (Wei et al. 1990; Wojdyło et al. 2007; Kaur et al. 2008; Pejin et al. 2013; Salehi et al. 2019). However, their nephroprotective effect has not been explored yet. Therefore we hypothesised that these dietary substances show nephroprotective effect against cisplatin-induced renal injury using human embryonic kidney (HEK-293) cells as our in-vitro model system.

2. Results and discussion
2.1. Effect of API, KMP and CIS on cellular proliferation and toxicity

The effect of API and KMP alone and in combination with CIS treatment on cellular proliferation was assessed by MTT assay. Various concentration of compounds i.e., API & KMP (0, 1.56, 3.125, 6.25, 12.5, 25 μg/mL) and CIS (0, 5, 10, 20, 40 μM) were used to treat HEK 293 cells for 48 h. As shown in Figure S1 A(i), CIS was found to significantly inhibit the proliferation of HEK-293 cells with half-maximal inhibitory concentration (IC50) values of 11.36 μM. Further, the effects of API and KMP treatment on HEK-293 cellular proliferation were also investigated, however no substantial reduction of cellular proliferation was observed up to 12.5 μg/mL API and 25 μg/mL KMP concentrations as shown in Figure S1 A (ii).

Additionally, HEK-293 cells were treated with 11.36 μM of cisplatin for 48 h in the absence or presence of a varying concentration of API & KMP, and then cell morphology was observed to evaluate the protective effect of compounds on cisplatin-induced cytotoxicity. After exposure to cisplatin, HEK-293 cells were damaged and a significant reduction of cell density was observed as shown in Figure S1 B-C. On the other hand, treatment with API and KMP reduced cisplatin-induced cellular damage, with no significant change in cell density with an increase in concentration Figure S1 B-C.

Taken together our findings show that CIS inhibits cellular proliferation of HEK-293 cells and causes cell toxicity, whereas API and KMP did not inhibit cellular proliferation of HEK-293 cells and were not determined to be cytotoxic at a concentration of 12.5 μg/mL and 25 μg/mL, respectively. Treatment with API and KMP in combination
with CIS, on the other hand, significantly reduced the cellular damage produced by CIS-induced cytotoxicity. Based on these findings, further experiments were performed to decipher the mechanism of the nephroprotective effect of API & KMP in combination with CIS.

2.2. Effect of API and KMP on CIS-induced oxidative stress on HEK-293 cells

One important mechanism of cisplatin-induced kidney injury is oxidative stress (I et al. 2004). Therefore, the effect of various concentrations of API and KMP (0, 1.56, 3.125, 6.25, 12.5, 25 µg/mL) in combination with CIS treatment were assessed for ROS generation using flow cytometry analysis. As depicted in Figure S2A-B, the highest generation of ROS was monitored after treating HEK-293 cells with cisplatin owing to the fluorescence intensity directly proportional to the amount of oxidised DCFDA to FCF form of the dye. On the contrary, treatment with API & KMP in combination with CIS reduced the CIS-induced oxidative stress at all the concentrations selected and showed the best activity at the highest concentration taken. Our findings show that at concentrations of 12.5 µg/mL API and 25 µg/mL KMP in combination with 11.36 µM of CIS, ROS production was decreased and therefore reduces CIS-induced oxidative stress. A plausible cause of this effect might be associated with the efficient antioxidant activities of API and KMP.

2.3. Effect of API and KMP on CIS-induced DNA damage on HEK-293 cell

Since DNA damage is another major mechanism by which cisplatin induces cellular toxicity, we further assess the effect of API and KMP treatment along with cisplatin on the DNA damage of HEK-293 cells. As shown in Figure S2 C-D treatment with CIS induce DNA tail formation resulting from DNA fragmentation. However, treatment with API & KMP in combination with CIS abrogates CIS-induced DNA damage. Collectively, our results suggest that mitigation of cellular integrity and oxidative stress due to CIS-induced damage in HEK-293 cells after treatment with API and KMP in combination with CIS was due to the protection of both the compounds from CIS-induce DNA damage.

2.4. Effect of API and KMP on CIS-induced deregulation of NGAL protein on HEK-293 cells

Limited studies thus far have systematically evaluated the in vitro performance of next-generation biomarkers such as NGAL for their potential to detect nephrotoxicity (JR et al. 1994) (JB and N 1997). The expression of these biomarkers significantly increases when cisplatin toxicity or any drug-induced toxicity is encountered by the kidney cells.

Therefore we investigated whether the mode of action of API and KMP is through the mitigation of these biological biomarkers. We found that cisplatin treatment induced the expression of NGAL in HEK –293 cells while the treatment of API and KMP drastically reduced the cisplatin-induced expression of NGAL in HEK-293 cells.
This series of events suggest that API and KMP are guarding the kidney cells against cisplatin-induced toxicity when supplemented along with cisplatin.

3. Conclusions

The findings of the present study reveal that Apigenin and kaempferol, two dietary supplements, effectively ameliorates cisplatin-induced nephrotoxicity in kidney cells. Furthermore, the study provides corroborative scientific evidence and validates the medicinal use of dietary compounds as a renoprotective agent. The observed nephroprotective effect was associated with amelioration of nephroprotective protein NGAL and subsequent, DNA damage effect, ROS generation and cellular viability. However, whether this is a cause or a result of protection has yet to be confirmed. Collectively, apigenin and kaempferol hold the potential of being developed as a nephroprotective drug that could be used as adjuvant cancer treatment and guard against cisplatin-induced renal injury.

Acknowledgements

We acknowledge our host institute, B.V. Patel Pharmaceutical Education Research and Development (PERD) Centre, to provide us with the facilities to work.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

Author A. S. express gratitude to the Department of Science & Technology (DST), Ministry of Science and Technology, Government of India for INSPIRE fellowship (Grant no. IF190211).

ORCID

Abhilasha Sharma http://orcid.org/0000-0002-2438-6556
Neeta Shrivastava http://orcid.org/0000-0002-7600-6682

References

Arany I, Megyesi JK, Kaneto H, Price PM, Safirstein RL. 2004. Cisplatin-induced cell death is EGFR/src/ERK signaling dependent in mouse proximal tubule cells. Am J Physiol Renal Physiol. 287(3):F543–F549. [Internet]. [accessed 2021 Oct 17]. https://doi.org/10.1152/AJPRENAL.00112.2004.

Bundgaard JR, Sengeløv H, Bundgaard N, Kjeldsen L. 1994. Molecular cloning and expression of a cDNA encoding NGAL: a lipocalin expressed in human neutrophils. Biochem Biophys Res Commun. 202(3):1468–1475. [Internet]. [accessed 2021 Oct 17].

Cowland JB, Borregaard N. 1997. Molecular characterization and pattern of tissue expression of the gene for neutrophil gelatinase-associated lipocalin from humans. Genomics. 45(1):17–23. [Internet]. [accessed 2021 Oct 17].
Dasari S, Tchounwou PB. 2014. Cisplatin in cancer therapy: molecular mechanisms of action. Eur J Pharmacol. 740:364–378.

Effects and side-effects of cisplatin. 1982. Lancet. 1(8273):682.

Filipski KK, Mathijsen RH, Mikkelsen TS, Schinkel AH, Sparreboom A. 2009. Contribution of organic cation transporter 2 (OCT2) to cisplatin-induced nephrotoxicity. Clin Pharmacol Ther. 86(4):396–402.

Florea A-M, Büsselberg D. 2011. Cisplatin as an anti-tumor drug: cellular mechanisms of activity, drug resistance and induced side effects. Cancers (Basel). 3(1):1351–1371.

Gonzalez-Vitale JC, Hayes DM, Cvitkovic E, Sternberg SS. 1977. The renal pathology in clinical trials of cis-platinum (II) diaminedichloride. Cancer. 39(4):1362–1371.

Kaur P, Shukla S, Gupta S. 2008. Plant flavonoid apigenin inactivates Akt to trigger apoptosis in human prostate cancer: an in vitro and in vivo study. Carcinogenesis. 29(11):2210–2217.

Pabla N, Murphy RF, Liu K, Dong Z. 2009. The copper transporter Ctr1 contributes to cisplatin uptake by renal tubular cells during cisplatin nephrotoxicity. Am J Physiol Renal Physiol. 296(3):F505–11.

Pejin B, Bogdanovic-Pristov J, Pejin I, Sabovljevic M. 2013. Potential antioxidant activity of the moss Bryum moravicum. Nat Prod Res. 27(10):900–902. [Internet].

Pierson-Marchandise M, Gras V, Moragny J, Micallef J, Gaboriau L, Picard S, Choukroun G, Masmoudi K, Liabeuf S, French National Network of Pharmacovigilance Centres 2017. The drugs that mostly frequently induce acute kidney injury: a case - noncase study of a pharmacovigilance database. Br J Clin Pharmacol. 83(6):1341–1349.

Salehi B, Venditti A, Sharifi-Rad M, Kregiel D, Sharifi-Rad J, Durazzo A, Lucarini M, Santini A, Souto E, Novellino E, et al. 2019. The therapeutic potential of apigenin. UMS. [Internet]. 20(6):1305.

Siddik ZH. 2003. Cisplatin: mode of cytotoxic action and molecular basis of resistance. Oncogene. 22(47):7265–7279.

Wei H, Tye L, Bresnick E, Birt DF. 1990. Inhibitory effect of apigenin, a plant flavonoid, on epidermal ornithine decarboxylase and skin tumor promotion in mice. Cancer Res. 50(3):499–502.

Wojdylo A, Oszmianski J, Czemerys R. 2007. Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chem . 105(3):940–949. [Internet].

Zamble DB, Lippard SJ. 1995. Cisplatin and DNA repair in cancer chemotherapy. Trends Biochem Sci. 20(10):435–439.