Investigational
Medicinal Chemistry & Pharmacology

Research Article Open Access

Catechin Derivatives from *Parkia biglobosa* Displayed Selective Cytotoxicity Towards Leukemia CCRF-CEM Cell Line and its P-Glycoprotein Expressing Subline CEM/ADR5000

Victor Kuete[1,2*], Viviane R. Sipowo Tala[3,4], Armelle T. Mbaveng[2], Viviane Cândida da Silva[4], Clenilson M. Rodrigues[4,5], Augustin E. Nkengfack[6], Lourdes Campaner dos Santos[4] ,Wagner Vilegas[4,7] and Thomas Efferth[1*]

Abstract

Background: Cancer chemotherapy is challenged by drug resistance of malignant cells. In the present work, we evaluated the cytotoxicity of four catechin derivatives, 4'-methylepigallocatechin (**1**), gallocatechin (**2**), epigallocatechin (**3**), epigallocatechin-3-*O*-gallate (**4**) against nine drug sensitive and multidrug resistant (MDR) cancer cell lines.

Methods: The resazurin reduction assay was used to evaluate the cytotoxicity of the compounds.

Results: Catechin derivatives **1-4** displayed selective cytotoxic effect with IC_{50} values below 90 µM on the leukemia CCRF-CEM cells and it drug resistant subline CEM/ADR5000. In contrast, no IC_{50} values could be measured in all 7 carcinoma cell lines tested. CEM/ADR5000 cells were much more cross-resistant to doxorubicin than to compounds **1-4**.

Conclusions: Hence, the four compounds may serve as lead drugs for further derivatization and can be explored in more details for their possible use to treat leukemia.

Keywords: catechin; cytotoxicity; epigallocatechin-3-*O*-gallate; multidrug resistance; leukemia.

Background

Cancer is the leading cause of morbidity and mortality worldwide and accounts for 12.5% deaths [1, 2]. It is estimated that the number of cancer deaths will reach 11.5 million in 2030 [3]. Globally, cancer is the second leading cause of death amongst non-communicable diseases, after cardiovascular diseases [2]. Cancer chemotherapy is challenged by drug resistance of malignant cells and toxicity of antineoplastic agents. This propels the search of new anticancer drugs with low toxicity and able to combat multidrug resistant (MDR) cell lines. In the past, plants have yielded several anticancer agents used in chemotherapy such as vinblastine, vincristine, vindesine, etoposide, teniposide, paclitaxel, docetaxel, camptotecin and irinotecan etc [2]. the

*Correspondence: *kuetevictor@yahoo.fr (Prof. Dr. Victor Kuete); efferth@uni-mainz.de (Prof. Dr. Thomas Efferth)*
[1]Department of Pharmaceutical Biology, Institute of Pharmacy and Biochemistry, University of Mainz, Staudinger Weg 5, 55128 Mainz, Germany; [2]Department of Biochemistry, Faculty of Sciences, University of Dschang, Cameroon; [3]Faculty of Health Sciences, Université des Montagnes, Cameroon; [4] UNESP—Sao Paulo State University, Department of Organic Chemistry, Institute of Chemistry14800-900, Araraquara, Sao Paulo, Brazil; [5]Embrapa Agroenergy, PqEB, W3 Norte, 70770-901, Brasilia, Distrito Federal, Brazil; [6]Department of Organic Chemistry, Faculty of Sciences, University of Yaounde I, Cameroon;[7]UNESP—Sao Paulo State University, Campus Coastal of São Vicente, 11350-000, Sao Vicente, Sao Paulo, Brazil;

Citation on this article: Kuete V, Sipowo Tala VR, Mbaveng AT, Cândida da Silva V, Rodrigues CM, Nkengfack AE, dos Santos LC , Vilegas W and Efferth T. Investigational Medicinal Chemistry and Pharmacology (2018) 1(1):2

plant Kingdom is still a valuable source of anticancer agents . During the past five years, we reported a panel of prominent cytotoxic compounds from African medicinal plants [4]. Some of them include benzophenones [5], favonoids [6-8], xanthones [9, 10], isoflavonoids [11], alkaloids [12] etc. In our continous search for cytotoxic leads from medicinal plants, we herein targeted catechin derivatives. Catechins are polyphenolic compounds found at high concentrations in a variety of plants. Catechins are classified as flavanols and they can include afzelechin, catechin, epicatechin, epigallocatechin, epicatechin gallate, and epigallocatechin gallate either in its monomeric or oligomeric forms [13]. A variety of biological activity was assigned to catechin. It was reported to induce longevity in the nematode worm *Caenorhabditis elegans* [14], to reduce atherosclerotic lesion development in mice [15], to inhibit intestinal tumor formation in mice [16], to inhibit the oxidation of low density lipoprotein [17]. (-)-Epicatechin was reported to protect the brain against intracerebral hemorrhage by activation of Nrf2-dependent and -independent pathways [18]. The present report was designed to evaluate the cytotoxicity of four catechin derivatives, 4'-methylepigallocatechin (**1**), gallocatechin (**2**), epigallocatechin (**3**), epigallocatechin-3-*O*-gallate (**4**) towards sensitive and drug-resistant leukemia and carcinoma cell lines.

Methods

Chemicals

The compounds used included 4'-methylepigallocatechin (**1**), gallocatechin (**2**), epigallocatechin (**3**), epigallocatechin-3-*O*-gallate (**4**) (Figure 1) previously isolated from *Parkia biglobosa* (Jacq.) G. Don. (Fabaceae) [19]. Doxorubicin 98.0% was provided by the University Pharmacy of the Johannes Gutenberg University (Mainz, Germany) and dissolved in PBS (Invitrogen, Eggenstein, Germany) at a concentration of 10 mM. Geneticin >98% was purchased from Sigma-Aldrich (Taufkirchen, Germany) and stored at a stock concentration of 72.18 mM.

Cell cultures

The cell lines used in the present work, their origins and their treatments were previously reported. They include drug-sensitive CCRF-CEM and multidrug-resistant P-glycoprotein-over-expressing CEM/ADR5000 leukemia cells [20-22], MDA-MB-231-pcDNA3 breast cancer cells and its resistant and transfectant subline MDA-MB-231-*BCRP* clone 23 [23], HCT116 ($p53^{+/+}$) colon cancer cells and its knockout clone HCT116 ($p53^{-/-}$), U87MG glioblastoma cells and its resistant and transfected subline U87MG.Δ*EGFR* [5, 10, 24].

Resazurin reduction assay

The cytotoxicity testing was performed by using the resazurin reduction assay as previously described [5, 10, 24-27].

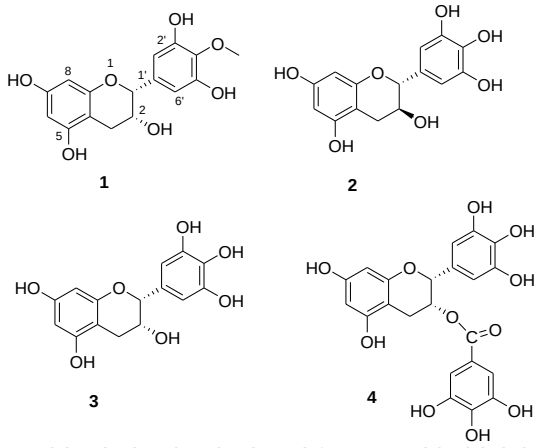

Fig.1. Chemical structures of the catechin derivative isolated from *Parkia biglobasa.***1:** 4'-methylepigallocatechin; **2:** gallocatechin; **3:** epigallocatechin; **4:** epigallocatechin-3-*O*-gallate

Results

The four tested catechin derivatives included 4'-methylepigallocatechin $C_{16}H_{16}O_7$ (**1**; *m/z* 320), gallocatechin $C_{15}H_{14}O_7$ (**2**; *m/z* 306), epigallocatechin $C_{15}H_{14}O_7$ (**3**; *m/z* 306), epigallocatechin-3-O-gallate $C_{22}H_{18}O_{11}$ (**4**; *m/z* 458) previously isolated from *Parkia biglobosa* (Jacq.) G. Don. (Fabaceae) [19].

These compounds were screened for their cytotoxicity using the resazurin assay and the results are summarized in Table 1. The degrees of resistance were determined as the ratio of IC_{50} value of the resistant cell line to that of the corresponding parental sensitive counterpart. As shown in Table 1, the four tested catechin derivatives displayed cytotoxic effects with IC_{50} values below 90 µM on the

two leukemia cell lines. In contrast, no IC_{50} values were obtained for all the seven carcinoma cell lines at concentrations up to 100 µM. The reference drug displayed IC_{50} values ranged from 0.20 µM (against CCRF-CEM cells) and 195.12 µM (against CEM/ADR5000 cells). No IC_{50} values were obtainable when compounds **1** were tested (> 125.39 µM), as well as **2** and **3** (>131.15 µM) and **4** (> 87.53 µM) on AML12 normal hepatocytes. Cross-resistance to compounds **1-4** was observed with in P-glycoprotein-overexpressing CEM/ADR5000 cells (degree of resistance 2.06-fold to 2.77-fold) compared to their sensitive counterparts CCRF-CEM cells. However, CEM/ADR5000 cells were much more cross-resistant to doxorubicin (>975.60-fold).

Table 1. Cytotoxicity of four catechin derivatives from *Parkia biglobasa* and doxorubicin towards sensitive and drug-resistant cancer cell lines as well as normal cells as determined by the resazurin assay.

Cell lines	Samples, IC_{50} values in µM and degrees of resistance* (in bracket)				
	Compounds				Doxorubicin
	1	**2**	**3**	**4**	
CCRF-CEM	26.93±1.73	21.93±1.16	41.80±3.94	**9.72±0.76**	**0.20±0.06**
CEM/ADR5000	74.70±6.42	52.16±5.07	90.03±6.85	20.07±3.15	195.12±14.30
Degree of resistance*	(2.77)	(2.38)	(2.15)	(2.06)	(975.60)
MDA-MB-231-*pcDNA*	>125.39	>131.15	>131.15	>87.53	**1.10±0.28**
MDA-MB-231-*BCRP*	>125.39	>131.15	>131.15	>87.53	**7.83±0.47**
Degree of resistance					(7.12)
HCT116 ($p53^{+/+}$)	>125.39	>131.15	>131.15	>87.53	**1.41±0.29**
HCT116 ($p53^{-/-}$)	>125.39	>131.15	>131.15	>87.53	**4.06±0.07**
Degree of resistance					(2.88)
U87MG	>125.39	>131.15	>131.15	>87.53	**1.06±0.15**
U87MG.Δ*EGFR*	>125.39	>131.15	>131.15	>87.53	**6.11±0.57**
Degree of resistance					(5.76)
HepG2	>125.39	>131.15	>131.15	>87.53	**3.83±0.94**
AML12	>125.39	>131.15	>131.15	>87.53	>73.59
Degree of resistance					(> 19.00)

(*): The degree of resistance was determined as the ratio of IC_{50} value in the resistantdivided by the IC_{50} in the sensitive cell line; AML12, CEM/ADR5000, MDA-MB-231-*BCRP*, HCT116 ($p53^{-/-}$) and U87MG.Δ*EGFR* were used as the corresponding resistant counterpart for CCRF-CEM, MDA-MB-231-*pcDNA*, HCT116 ($p53^{+/+}$), U87MG and HepG2 respectively; **1:** 4'-methylepigallocatechin; **2:** gallocatechin; **3:** epigallocatechin; **4:** epigallocatechin-3-*O*-gallate

Discussion

The search of new compounds with efficiency against drug-sensitive and drug-resistant cancer cells lines is very important in the global fight against neoplastic diseases. Reporting on compounds with prominent data is also as important as reporting on negative results, to avoid other authors to perform useless experiments. In the present study, we tested the ability of catechin derivatives to prevent the growth of both sensitive and resistant cancer cells. According to the established criteria, an IC_{50} value threshold of 4 µg/mL or 10 µM [28, 29] after 48 and 72 h incubations has been set to identify good cytotoxic compounds. The activity is moderate, if $10<IC_{50}<50$ µM [4]. In this study, IC_{50} value of 9.72 µM was obtained only with compound **4** in the sensitive CCRF-CEM leukemia cells. IC_{50} values above 10 µM were obtained in CEM/ADR5000 leukemia cells while no obtainable value was recorded on carcinoma cells. This suggests that the four catechin derivatives could not be considered as good anticancer agents to fight solid tumors.

Though, the cytotoxicity of catechin was reported in several cancer cells *in vivo* [30], this study rather shows that its derivatives are poor cytotoxic agents *in vitro*. To the best of our knowledge, the cytotoxicity of catechin derivatives **1-4** in the studied cancer cell lines is being reported for the first time. Nonetheless, the poor activity of catechin derivatives in other carcinoma cells was previously reported. In fact, epigallocatechin-3-*O*-gallate (**4**) displayed poor *in vitro* cytotoxic effects towards glioma cell lines 1321N1 (IC_{50} of 82 µg/mL), SW1783 (IC_{50}: 300 µg/mL) and LN18 (IC_{50}: 134 µg/mL) [31]. Epigallocatechin gallate is the major catechin component in green tea and it appears to be the most biologically active one [32]. Among the beneficial effects of tea polyphenols, its cancer preventive activity has been reported [30]. Many studies have demonstrated the modulation of signal transduction

and metabolic pathways by epigallocatechin-3-*O*-gallate (**4**), the most abundant and active polyphenol in green tea [30]. In the present study, the cytotoxicity of compound **4** was good towards the sensitive CCRF-CEM leukemia cells and moderate towards its reistant subline CEM/ADR5000 (Table 1). However, this compound did not show any cytotoxicity towards carcinoma cells. It was demonstrated that compound **4** inhibited tumorigenesis in animal models [30]. Though *in vivo* effects towards carcinoma cells have been reported, we did not observe promising *in vitro* activity herein. The *in vivo* effects of **4** might be due to other anticancer mechanisms such as inhibition of angiogenesis, as this was also reported to be one of the mode of action of green tea [33].

Conclusions

In conclusion, we demonstrated that catechin derivatives 4'-methylepigallocatechin (**1**), gallocatechin (**2**), epigallocatechin (**3**), epigallocatechin-3-*O*-gallate (**4**) were selectively active against leukemia cell lines. We also demonstrated that the tested compounds did not have appreciable *in vitro* cytotoxic effects on carcinoma cells. The four compounds can be therefore be explored in more detail for their possible use in the treatment of leukemia.

Authors' Contribution

VK out the experiments; VK, VRST, ATM, VCDS, CMR, AEN, LCDS, WV contributed to compound´s isolation and/or identification. VK wrote the manuscript. VK and TE designed the experiments; TE supervised the work and provided the facilities for the study. All authors read the manuscript and approved the final version.

Acknowledgments

VK is very grateful to the Alexander von Humboldt Foundation for an 18 months' fellowship in Germany through the "Georg Foster Research Fellowship for Experienced Researcher" program.

Conflict of interest

VK is the Editor-In-Chief of Investigational Medicinal Chemistry and Pharmacology; Other authors have no competing interest.

Article history:
Received : 5 January 2018
Received in revised form : 14 March 2018
Accepted : 22 May 2018
Available online : 23 May 2018

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