Резюмета на трудовете, след защита на докторска дисертация

представен от

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1. Multifunctional Pt(IV) prodrug candidates featuring the carboplatin core and deferoxamine. S. Harringer, M. Hejl, E. A. Enyedy, M. A. Jakupec, M. S. Galanski, B. K. Keppler, P. J. Dyson, **H. P. Varbanov***; *Dalton Trans.,* **2021**, 50, 8167-8178. **Q1**
https://doi.org/10.1039/D1DT00214G.

The synergistic combination of the anticancer drug carboplatin and the iron chelator deferoxamine (DFO) served as a foundation for the development of novel multifunctional prodrugs. Hence, five platinum(IV) complexes, featuring the equatorial coordination sphere of carboplatin, and one or two DFO units incorporated at axial positions, were synthesized and characterized using ESI-HRMS, multinuclear (^{1}H, ^{13}C, ^{15}N, ^{195}Pt) NMR spectroscopy and elemental analysis. Analytical studies demonstrated that the chelating properties of the DFO moiety were not compromised after coupling to the platinum(IV) core. The cytotoxic activity of the compounds was evaluated in monolayer (2D) and spheroid (3D) cancer cell models, derived from ovarian teratocarcinoma (CH1/PA-1), colon carcinoma (SW480) and non-small cell lung cancer (A549). The platinum(IV)–DFO prodrugs demonstrated moderate in vitro cytotoxicity (a consequence of their slow activation kinetics) but with less pronounced differences between intrinsically chemoresistant and chemosensitive cell lines as well as between 2D and 3D models than the clinically used platinum(II) drug carboplatin.

2. Screening-based approach to discover effective platinum-based chemotherapies for cancers with poor prognosis.

H. P. Varbanov*, F. Kuttler, D. Banfi, G. Turcatti, P. J. Dyson*; *PLoS ONE*, **2019**, 14(1): e0211268. **Q1**

https://doi.org/10.1371/journal.pone.0211268.

Drug combinations are extensively used to treat cancer and are often selected according to complementary mechanisms. Here, we describe a cell-based high-throughput screening assay for identification of synergistic combinations between broadly applied platinum-based chemotherapeutics and drugs from a library composed of 1280 chemically and pharmacologically diverse (mostly FDA approved) compounds. The assay was performed on chemoresistant cell lines derived from lung (A549) and pancreatic (PANC-1) carcinoma, where platinum-based combination regimens are currently applied though with limited success. The synergistic combinations identified during the screening were validated by synergy quantification using the combination index method and via high content fluorescent micros- copy analysis. New promising synergistic combinations discovered using this approach include compounds currently not used as anticancer drugs, such as cisplatin or carboplatin with hycanthone and cisplatin with spironolactone in pancreatic carcinoma, and carboplatin and deferoxamine in non-small cell lung cancer. Strong synergy between cisplatin or carboplatin and topotecan in PANC-1 cells, compared to A549 cells, suggests that this combination, currently used in lung cancer treatment regimens, could be applied to pancreatic carcinoma as well. Several drugs used to treat diseases other than cancer, including pyrvinium pamoate, auranofin, terfenadine and haloprogin, showed strong cytotoxicity on their own and synergistic interactions with platinum drugs. This study demonstrates that non-obvious drug combinations that would not be selected based on complementary mechanisms can be identified via high-throughput screening.

3. Development and validation of liquid chromatography-based methods to assess the lipophilicity of cytotoxic platinum(IV) complexes.

M. H. M. Klose, S. Theiner, **H. P. Varbanov**, D. Hoefer, V. Pichler, M. Galanski, S. M. Meier-Menches*, B. K. Keppler*; *Inorganics*, **2018**, 6(4), 130. (2018 *Inorganics* Best Paper Award) **Q3**

https://doi.org/10.3390/inorganics6040130.

Lipophilicity is a crucial parameter for drug discovery, usually determined by the logarithmic partition coefficient (Log P) between octanol and water. However, the available detection methods have restricted the widespread use of the partition coefficient in inorganic medicinal chemistry, and recent investigations have shifted towards chromatographic lipophilicity parameters, frequently without a conversion to derive Log P. As high-performance liquid chromatography (HPLC) instruments are readily available to research groups, a HPLC-based method is presented and validated to derive the partition coefficient of a set of 19 structurally diverse and cytotoxic platinum(IV) complexes exhibiting a dynamic range of at least four orders of magnitude. The chromatographic lipophilicity parameters φ_0 and Log k_W were experimentally determined for the same set of compounds, and a correlation was obtained that allows interconversion between the two lipophilicity scales, which was applied to an additional set of 34 platinum(IV) drug candidates. Thereby, a $\varphi_0 = 58$ corresponds to Log P = 0. The same approaches were successfully evaluated to determine the distribution coefficient (Log D) of five ionisable platinum(IV) compounds to sample pH-dependent effects on the lipophilicity. This study provides straight-forward HPLC-based methods to determine the lipophilicity of cytotoxic platinum(IV) complexes in the form of Log P and φ_0 that can be interconverted and easily expanded to other metal-based compound classes.

4. The impact of whole human blood on the kinetic inertness of platinum(IV) prodrugs – an HPLC-ICP-MS study.

S. Theiner, M. Grabarics, L. Galvez, **H. P. Varbanov**, N. S. Sommerfeld, M. Galanski, B. K. Keppler, G. Koellensperger*; *Dalton Trans.*, **2018**, 47, 5252-5258. **Q1** https://doi.org/10.1039/C7DT04537A.

The potential advantage of platinum(IV) complexes as alternatives to classical platinum(II)-based drugs relies on their kinetic stability in the body before reaching the tumor site and on their activation by reduction inside cancer cells. In this study, an analytical workflow has been developed to investigate the reductive biotransformation and kinetic inertness of platinum(IV) prodrugs comprising different ligand coordination spheres (respectively, lipophilicity and redox behavior) in whole human blood. The distribution of platinum(IV) complexes in blood pellets and plasma was determined by inductively coupled plasma-mass spectrometry (ICP-MS) after microwave digestion. An analytical approach based on reversed-phase (RP)-ICP-MS was used to monitor the parent compound and the formation of metabolites using two different extraction procedures. The ligand coordination sphere of the platinum(IV) complexes had a significant impact on their accumulation in red blood cells and on their degree of kinetic inertness in whole human blood. The most lipophilic platinum(IV) compound featuring equatorial chlorido ligands showed a pronounced penetration into blood cells and a rapid reductive biotransformation. In contrast, the more hydrophilic platinum(IV) complexes with a carboplatin- and oxaliplatin-core exerted kinetic inertness on a pharmacologically relevant time scale with notable amounts of the compound accumulated in the plasma fraction.

5. Impact of the equatorial coordination sphere on the rate of reduction, lipophilicity and cytotoxic activity of platinum(IV) complexes.

D. Höfer, **H. P. Varbanov**, M. Hejl, M. A. Jakupec, A. Roller, M. Galanski*, B. K. Keppler*; *J. Inorg. Biochem.*, **2017**, 174, 119–129. **Q2**

https://doi.org/10.1016/j.jinorgbio.2017.06.005.

The impact of the equatorial coordination sphere on the reduction behavior (i.e. rate of reduction) of platinum (IV) complexes with axial carboxylato ligands was studied. Moreover, the influence of equatorial ligands on the stability, lipophilicity and cytotoxicity of platinum(IV) compounds was evaluated. For this purpose, a series of platinum(IV) complexes featuring axial carboxylato ligands (succinic acid monoesters) was synthesized; anionic carboxylato (OAc^-, oxalate) and halido (Cl^-, Br^-, I^-) ligands served as leaving groups and am(m)ine carrier ligands were provided by monodentately (isopropylamine, ammine + cyclohexaneamine) or bidentately (ethane-1,2-diamine) coordinating am(m)ines. All platinum(IV) products were fully characterized based on elemental analysis, high resolution mass spectrometry and multinuclear (^{1}H, ^{13}C, ^{15}N, ^{195}Pt) NMR spectroscopy as well as by X-ray diffraction in some cases. The rate of reduction in the presence of ascorbic acid was determined by NMR spectroscopy and the lipophilicity of the complexes was investigated by analytical reversed phase HPLC measurements. Cytotoxic properties were studied by means of a colorimetric microculture assay in three human cancer cell lines derived from cisplatin sensitive ovarian teratocarcinoma (CH1/PA-1) as well as cisplatin insensitive colon carcinoma (SW480) and non-small cell lung cancer (A549).

6. Oxaliplatin reacts with DMSO only in the presence of water.

H. P. Varbanov*, D. Ortiz, D. Höfer, L. Menin, M. Galanski, B. K. Keppler, P. J. Dyson*; *Dalton Trans.*, **2017**, 46, 8929–8932. (with inner cover picture) **Q1**

https://doi.org/10.1039/c7dt01628j.

Herein we show that oxaliplatin reacts rapidly with DMSO in aqueous solutions, despite being stable in pure DMSO and pure water. Furthermore, the reactivity of the clinically applied Pt(II) drugs in water/DMSO and PBS/DMSO mixtures, and the nature of the species formed were investigated by MS, NMR and RP-HPLC techniques.

7. Repositioning approved drugs for the treatment of problematic cancers using a screening approach.

H. P. Varbanov*, F. Kuttler, D. Banfi, G. Turcatti, P. J. Dyson*; *PLoS ONE*, **2017**, 12(2): e0171052. **Q1**

https://doi.org/10.1371/journal.pone.0171052.

Advances in treatment strategies together with an earlier diagnosis have considerably increased the average survival of cancer patients over the last four decades. Nevertheless, despite the growing number of new antineoplastic agents introduced each year, there is still no adequate therapy for problematic malignancies such as pancreatic, lung and stomach cancers. Consequently, it is important to ensure that existing drugs used to treat other types of cancers, and potentially other diseases, are not overlooked when searching for new chemotherapy regimens for these problematic cancer types. We describe a screening approach that identifies chemotherapeutics for the treatment of lung and pancreatic cancers, based on drugs already approved for other applications. Initially, the 1280 chemically and pharma- cologically diverse compounds from the Prestwick Chemical Library® (PCL) were screened against A549 (lung cancer) and PANC-1 (pancreatic carcinoma) cells using the PrestoBlue fluorescent-based cell viability assay. More than 100 compounds from the PCL were identified as hits in one or both cell lines (80 of them, being drugs used to treat diseases other than cancer). Selected PCL hits were further evaluated in a dose-response manner. Promising candidates for repositioning emanating from this study include antiparasitics, cardiac glycosides, as well as the anticancer drugs vorinostat and topotecan.

8. The role of the equatorial ligands for the redox behavior, mode of cellular accumulation and cytotoxicity of platinum(IV) prodrugs.

S. Goeschl, **H.P. Varbanov***, S. Theiner, M.A. Jakupec*, M. Galanski, B.K. Keppler; *J. Inorg. Biochem.*, **2016***, 160,* 264-274. **Q2**

https://doi.org/10.1016/j.jinorgbio.2016.03.005.

The current study aims to elucidate the possible reasons for the significantly different pharmacological behavior of platinum(IV) complexes with cisplatin-, carboplatin- or nedaplatin-like cores and how this difference can be related to their main physicochemical properties. Chlorido-containing complexes are reduced fast (within hours) by ascorbate and are able to unwind plasmid DNA in the presence of ascorbate, while their tri- and tetracarboxylato analogs are generally inert under the same conditions. Comparison of the lipophilicity, cellular accumulation and cytotoxicity of the investigated platinum compounds revealed the necessity to define new structure-property/activity relationships (SPRs and SARs). The higher activity and improved accumulation of platinum(IV) complexes bearing Cl^- in equatorial position cannot only be attributed to passive diffusion facilitated by their lipophilicity. Therefore, further platinum accumulation experiments under conditions where active/ facilitated transport mechanisms are suppressed were performed. Under hypothermic conditions (4 °C), accumulation of dichloridoplatinum(IV) complexes is reduced down to 10% of the amount determined at 37 °C. These findings suggest the involvement of active and/or facilitated transport in cellular uptake of platinum(IV) complexes with a cisplatin-like core. Studies with ATP depletion mediated by oligomycin and low glucose partially confirmed these observations, but their feasibility was severely limited in the adherent cell culture setting.

9. Prediction of logP for Pt(II) and Pt(IV) complexes: Comparison of statistical and quantum-chemistry based approaches.

I.V. Tetko*, **H.P. Varbanov**, M. Galanski, M. Talmaciu, J.A. Platts, M. Ravera, E. Gabano; *J. Inorg. Biochem.*, **2016**, 156, 1-13. **Q2**

https://doi.org/10.1016/j.jinorgbio.2015.12.006.

The octanol/water partition coefficient, logP, is one of the most important physicochemical parameters for the development of new metal-based anticancer drugs with improved pharmacokinetic properties. This study ad- dresses an issue with the absence of publicly available models to predict logP of Pt(IV) complexes. Following data collection and subsequent development of models based on 187 complexes from literature, we validate new and previously published models on a new set of 11 Pt(II) and 35 Pt(IV) complexes, which were kept blind during the model development step. The error of the consensus model, 0.65 for Pt(IV) and 0.37 for Pt(II) complexes, indicates its good accuracy of predictions. The lower accuracy for Pt(IV) complexes was attributed to experimental difficulties with logP measurements for some poorly-soluble compounds. This model was developed using general-purpose descriptors such as extended functional groups, molecular fragments and E-state in- dices. Surprisingly, models based on quantum-chemistry calculations provided lower prediction accuracy. We also found that all the developed models strongly overestimate logP values for the three complexes measured in the presence of DMSO. Considering that DMSO is frequently used as a solvent to store chemicals, its effect should not be overlooked when logP measurements by means of the shake flask method are performed. The final models are freely available at http://ochem.eu/article/76903.

10. Multi-scale imaging of anticancer platinum(IV) compounds in murine tumor and kidney.
A.A. Legin, S. Theiner, A. Schintlmeister, S. Reipert, P. Heffeter, M.A. Jakupec, J. Mayr, **H.P. Varbanov**, C.R. Kowol, M. Galanski, W. Berger, M. Wagner, B.K. Keppler*; *Chem. Sci.*, **2016**, 7(5), 3052-3061. **Q1**
https://doi.org/10.1039/c5sc04383b.

Nano-scale secondary ion mass spectrometry (NanoSIMS) enables trace element and isotope analyses with high spatial resolution. This unique capability has recently been exploited in several studies analyzing the subcellular distribution of Au and Pt anticancer compounds. However, these studies were restricted to cell culture systems. To explore the applicability to the in vivo setting, we developed a combined imaging approach consisting of laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS), NanoSIMS and transmission electron microscopy (TEM) suitable for multi-scale detection of the platinum distribution in tissues. Applying this approach to kidney and tumor samples upon administration of selected platinum(IV) anticancer prodrugs revealed uneven platinum distributions on both the organ and subcellular scales. Spatial platinum accumulation patterns were quantitatively assessed by LA-ICP-MS in histologically heterogeneous organs (e.g., higher platinum accumulation in kidney cortex than in medulla) and used to select regions of interest for subcellular-scale imaging with NanoSIMS. These analyses revealed cytoplasmic sulfur-rich organelles accumulating platinum in both kidney and malignant cells. Those in the tumor were subsequently identified as organelles of lysosomal origin, demonstrating the potential of the combinatorial approach for investigating therapeutically relevant drug concentrations on a submicrometer scale.

11. Tetracarboxylatoplatinum(IV) complexes featuring monodentate leaving groups - A rational approach toward exploiting the platinum(IV) prodrug strategy.
D. Hoefer, **H.P. Varbanov**, A. Legin, M.A. Jakupec, A. Roller, M. Galanski*, B.K. Keppler*; *J. Inorg. Biochem.,* **2015**, 153, 259-271. **Q1**
https://doi.org/10.1016/j.jinorgbio.2015.08.018.

A series of novel symmetrically and unsymmetrically coordinated platinum(IV) complexes with monodentate carboxylato ligands was synthesized. The compounds exhibit a general coordination sphere of [Pt(en)$(OCOR)_2$(OCOR′)(OCOR″)], where the carboxylato ligands are represented by acetato and succinic acid monoester ligands. Dicarboxylatoplatinum(II) complexes were synthesized and oxidized symmetrically or unsymmetrically to obtain platinum(IV) complexes, which were subsequently carboxylated with noncyclic anhydrides. The compounds were investigated in detail by elemental analysis, mass spectrometry, infrared and multinuclear (^{1}H, ^{13}C, ^{15}N, ^{195}Pt) NMR spectroscopy as well as by X-ray diffraction in some cases. The reduction behavior was followed by NMR spectroscopy, while stability and lipophilicity were examined by analytical reversed phase HPLC measurements. Cytotoxic properties were studied in three human cancer cell lines derived from cisplatin sensitive ovarian teratocarcinoma (CH1/PA-1), cisplatin insensitive colon carcinoma (SW480) and non-small cell lung cancer (A549). Thereby, the most lipophilic (yet water soluble) platinum(IV) complexes showed promising IC_{50} values in the low micromolar and even nanomolar range, demonstrating the significant advantage of using equatorially coordinated monodentate carboxylato ligands.

12. Tumor microenvironment in focus: LA-ICP-MS bioimaging of a preclinical tumor model upon treatment with platinum(IV)-based anticancer agents.
S. Theiner, C. Kornauth, **H. P. Varbanov**, M. Galanski, S. Schoonhoven, P. Heffeter, W. Berger, A. E. Egger*, B. K. Keppler; *Metallomics*, **2015**, 7(8), 1256-1264. **Q1** https://doi.org/10.1039/c5mt00028a.

The selection of drug candidates for entering clinical development relies on in vivo testing in (solid) tumor animal models. However, the heterogeneity of tumor tissue (e.g. in terms of drug uptake or tissue composition) is rarely considered when testing novel drug candidates. Therefore, we used the murine colon cancer CT-26 tumor model to study the spatially-resolved drug distribution in tumor tissue upon repetitive treatment of animals over two weeks with three investigational platinum(IV)-based anticancer agents, oxaliplatin or satraplatin. A quantitative laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) imaging method revealed a heterogeneous platinum distribution, which correlated well with the histologic features of the tumor and surrounding tissue at the microscopic level. In most of the cases, higher amounts of intratumoral platinum were found in the surrounding tissue than in the malignant parts of the sample. This indicates that determination of average platinum amounts (e.g. by microwave-assisted digestion of the sample followed by analysis with ICP-MS) might overestimate the drug uptake in tumor tissue causing misleading conclusions. In addition, we studied the platinum distribution in the kidneys of treated animals to probe if accumulation in the cortex and medulla predict potential nephrotoxicity. A 10-fold increase of platinum in the cortex of the kidney over the medulla was observed for oxaliplatin and satraplatin. Although these findings are similar to those in the platinum distribution of the nephrotoxic anticancer drug cisplatin, treatment with the compounds of our study did not show signs of nephrotoxicity in clinical use or clinical trials (oxaliplatin, satraplatin) and did not result in the alteration of renal structures. Thus, predicting the side effects based on bioimaging data by LA-ICP-MS should be considered with caution. To the best of our knowledge, this is the first LA-ICP-MS study on spatially- resolved platinum accumulation in tissues after repetitive platinum-based anticancer drug treatment of mice bearing a preclinical tumor model.

13. Comparative in vitro and in vivo pharmacological investigation of platinum(IV) complexes as novel anticancer drug candidates for oral application.

S. Theiner, **H. P. Varbanov**, M. Galanski, A. E. Egger, W. Berger, P. Heffeter*, B. K. Keppler*; *J. Biol. Inorg. Chem.*, **2015**, 20(1), 89-99. **Q1**

https://doi.org/10.1007/s00775-014-1214-6.

Platinum(IV) complexes are promising candidates as prodrugs for oral application in anticancer chemotherapy. However, only a few Pt(IV) compounds entered (pre)clinical trials, e.g. satraplatin, while most of the others were only tested in vitro. Aim of the study was investigation of the in vivo pharmacological behavior as well as the anticancer activity of two novel platinum(IV) complexes vs. satraplatin. The drugs were selected due to significantly different in vitro cytotoxicity while sharing some physicochemical properties (e.g. lipophilicity). Initial experiments indicated that the highly in vitro cytotoxic compound **1** ((OC-6-33)-dichloridobis((4-ethoxy)-4-oxobutanoato)-bis(ethylamine)platinum(IV)) was also characterized by high drug absorption and tissue platinum levels after oral application. Interestingly, analysis of serum samples using SEC-ICP-MS revealed that the administered drugs have completely been metabolized and/or bound to proteins in serum within 2 h after treatment. With regard to the activity in vivo, the outcomes were rather unexpected: although potent anticancer effect of **1** was observed in cell culture, the effects in vivo were rather minor. Nevertheless, **1** was superior to **2 (**(OC-6-33)-diammine(cyclobutane-1,1-dicarboxylato)-bis((4- cyclopentylamino)-4-oxobutanoato)platinum(IV)) after i.p. administration, which was, at least to some extent, in accordance to the cell culture experiments. After oral gavage, both compounds exhibited comparable activity. This is remarkable considering the distinctly lower activity of **2** in cell culture as well as the low platinum levels detected both in serum and tissues after oral application. Consequently, our data indicate that the prediction of in vivo anticancer activity by cell culture experiments is not trivial, especially for orally applied drugs.

14. A novel class of bis- and tris-chelate diam(m)inebis(dicarboxylato)platinum(IV) complexes as potential anticancer prodrugs.

H. P. Varbanov, S. Göschl, P. Heffeter, S. Theiner, A. Roller, F. Jensen, M. A. Jakupec, W. Berger, M. Galanski*, B. K. Keppler*; *J. Med. Chem.*, **2014**, 57(15), 6751-6764. **Q1** https://doi.org/10.1021/jm500791c.

A novel class of platinum(IV) complexes of the type [Pt(Am)($R(COO)_2)_2$], where Am is a chelating diamine or two monodentate am(m)ine ligands and $R(COO)_2$ is a chelating dicarboxylato moiety, was synthesized. For this purpose, the reaction between the corresponding tetrahydroxidoplatinum(IV) precursors and various dicarboxylic acids, such as oxalic, malonic, 3-methylmalonic, and cyclobutanedicarboxylic acid, was utilized. All new compounds were characterized in detail, using 1D and 2D NMR techniques, ESI-MS, FTIR spectroscopy, elemental analysis, TGA, and X-ray diffraction. Their in vitro cytotoxicity was determined in a panel of human tumor cell lines (CH1, SW480 and A549) by means of the MTT colorimetric assay. Furthermore, the lipophilicity and redox properties of the novel complexes were evaluated in order to better understand their pharmacological behavior. The most promising drug candidate, **4b** (Pt(DACH)$(mal)_2$), demonstrated low in vivo toxicity but profound anticancer activity against both the L1210 leukemia and CT-26 colon carcinoma models.

15. Design, synthesis and comparative cytotoxic investigation of platinum(II) complexes with some derivatives of 5-methyl- 5-(4-pyridyl)hydantoin.

A. Bakalova*, R. Buyukliev, **H. Varbanov**, G. Momekov, *Inorg. Chim. Acta*, **2014**, 423, 46-51. **Q2**

https://doi.org/10.1016/j.ica.2014.07.030.

A series of Pt(II) complexes with 3-ethyl-5-methyl-5-(4-pyridyl)hydantoin, 3-propyl-5-methyl-5-(4-pyridyl)hydantoin and 3-benzyl-5-methyl-5-(4-pyridyl)hydantoin with general formulae cis-$[Pt(L)_2Cl_2]$, cis- $[Pt(NH_3)LCl_2]$ and trans-$[Pt(NH_3)LCl_2]$ were synthesized. The new compounds were characterized by means of elemental analysis, IR, ^1H and ^{13}C NMR spectroscopy. The studies showed that the ligands coordinate to the platinum ions in a monodentate manner through the nitrogen atom from the pyridine ring. The cytotoxic activity in vitro of the complexes as well as of their previously prepared Pt(II) analogues with other derivatives of 5-methyl-5-(4-pyridyl)hydantoin was screened against a panel of human tumor cell lines. Cytotoxicity was strongly dependent on their lipophilicity, while the most lipophilic Pt(II) complex, carrying 3-benzyl-5-methyl-5-(4-pyridyl)hydantoin as carrier ligand inhibited the viability of tested cells at low micromolar concentrations with IC_{50} values comparable to that of cisplatin. A preliminary pharmacodynamic investigation showed that its cytotoxicity is mediated through induction of apoptosis. The cis- and trans-analogues consisting one ammine group in the molecules exhibited far less cytotoxicity in corroboration to the well-established structure–activity rules for dia(m)mine platinum(II) complexes.

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