Ingenious Dual Pulse Photoacoustic Tomography in Drug-Responsive Clinical Imaging Perspectives

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Abstract

Detection of the real-time growth rate of cancer and visualization of the effectiveness of chemotherapy using the live cell imaging technique are yet to be invented though these could be conducive to monitoring the cancer treatment more precisely. In the present article, a new technique is proposed that will be able to accomplish the aforementioned necessity. The recent development and success of the use of organometallic carbonyl clusters as photoacoustic contrast agents and cancer drugs have fetched a few freedom for the fate of new technologies towards the invention of time-dependent photoacoustic tomography (TD-PAT) that could ease the detection and treatment of cancer using advanced clinical chemotherapy.

Introduction

Instant detection of the growth rate of cancer in a living body is yet to be possible. The same is true for the advancement of success or failure of therapeutic treatment of cancer. Though

the growth rate of cancer may be estimated from different live cell imaging techniques at significant time intervals, live monitoring of chemotherapy could not be possible employing any available technique. The invention of a new technique that could be effective for live monitoring of cancer treatment will certainly increase the success ratio of cancer treatment. Is it possible? Yes, it is possible. How could it be possible?

To make the impossible possible, let us dream of an instrument that is capturing every successful action of a drug molecule. Thus, from the ratio of effective drug molecules and the total drug molecules, the effectiveness or success rate of the drug could be measured. To fulfill our dream, we have to apply only those drugs for the therapy which must act as exogenic contrast agents before and after binding to the respective enzyme or ligand to produce compounds having optical absorbance in two different regions such that both give effective contrast behaviors required for live cell imaging. The quest for cancer drug molecules having the aforementioned virtues is reported in the present communication.

Photoacoustic tomography (PAT)[1,2] is a widely used effective imaging technique today due to its deeper tissue penetration possibilities and high spatial resolution capability for live cell imaging.[3–8] The application of effective exogenous contrast agents[9] not only enhance the signal to noise ratio but also open up other important possibilities. Thus, most of the cell imaging techniques are implemented with bio-compatible contrast compounds. The same contrast agents may be applicable for different imaging techniques like MRI,[10] PAT,[11] CT scan,[12] USG,[13] etc. On the other hand, different classes of contrast media may also be implemented for the same technique.

For example, different classes of contrast agents like gold nanoparticles,,[14,15] organic dyes,[16–18] organometallic carbonyl clusters,[19–22] nanodyes,[23] etc have been tested for PAT so far. There are advantages as well as disadvantages one over another. However, the organometallic carbonyl cluster compounds of group-8 elements have attracted major attention due to their excellent solubility in water as well as hydrophobic media and high stability in blood circulation. These compounds have negligible cytotoxicity.[24–26] Interestingly, in a

number of recent studies it is reported that these compounds have remarkable anti-angiogenic activity too.[27] Thus, these compounds are chosen for our present study. If they exhibit optical absorbance properties at two different regions in the UV-Vis spectra before and after their addition to the respective enzyme or ligand to produce a detectable PAT signal, these could be our compound of the dream to design the time-dependent photoacoustic tomography (TD-PAT) mentioned earlier.

Recent advances in the field of ruthenium-based anti-cancer drugs including ruthenium carbonyl clusters give rise to the thinking of new medicinal technologies like time-dependent photoacoustic tomography (TD-PAT) which may be effective for the visualization of chemotherapeutic progress in a living body. The advantages and benefits of exploiting ruthenium as anti-cancer drugs have been discussed in a number of excellent reviews.[28–30] Indeed, ruthenium complexes are less cytotoxic than the worldwide approved platinum-based drugs due to the ability of ruthenium to mimic iron in binding to biological molecules, such as human serum albumin and transferrin.[31] Two ruthenium compounds, imidazolium trans- [tetrachlorobis (1H- indazole) ruthenate(III)]), termed KP1019,[32] and imidazolium trans- [tetrachloro(dimethyl sulfoxide) (1H-imidazole) ruthenate(III)]), termed NAMI-A,[33] are presently in phase II clinical trials, the latter compound showing both anti-metastatic and anti-angiogenic activity in preclinical models.[27] Organometallic ruthenium(II) complexes, [Ru(η^6 -arene)Cl_2 (PTA)] (arene = toluene and *p*-cymene, PTA = 1,3,5-triaza-7-phosphaadamantane), also exhibit anti-metastatic[34] and anti-angiogenic[35] properties.

Nazarov *et. al.*[27] showed that triruthenium-carbonyl clusters derivatized with glucose-modified bicyclophosphite ligands [Ru_3 $(CO)_x$ P_y, where P is one glucose-modified bicyclophosphite ligand] have very good anti-cancer activities with excellent cell uptake properties. They have shown that this compound shows angiogenic activities in vivo conditions by arresting the cell cycle in the G1/G0 phase which leads to cell apoptosis. Later, Kong *et. al.*[21] reported that triruthenium-carbonyl clusters derivatized with sodium thio-propanoate are quite good photoacoustic contrast agents. From these two successful studies, it could

be concluded that if triruthenium-carbonyl clusters are derivatized with a suitable organic ligand, they could act as the cancer drug and their drug activity could be followed using photoacoustic imaging. In the present research work, these possibilities are tested with a triruthenium-carbonyl cluster derivatized with glucose-modified bicyclophosphite ligands [Ru_3 $(CO)_{11}$ P].

Methods and Design

Design of TD-PAT

In photoacoustic tomography, a short pulse of electromagnetic radiation is used which is absorbed by some entities that exist in the living cell. Due to the absorption of the radiation heat is generated which creates acoustic waves. These waves travel to the tissue surface where a respective detector is situated. From the time delay to reach the detector, the spatial distribution of the absorbing entity could be imaged. The application of exogenous contrast agents enhances the absorption capacity and increases the photoacoustic signal. In the cancer-affected organs, the density of blood vessels is higher and hence the blood circulation. Thus, the use of exogenous contrast agents is very effective to differentiate normal cells from cancer cells. If the contrast agent itself is a cancer drug, then its implementation as a contrast agent not only enhances the signal resolution but also gives information about the status of the disease. The interaction of contrast agents with the cell may lead to cell apoptosis. Here two different situations may arise.

After the drug action, the drug molecule (in this case - the contrast agents) may lose its identity. The contrasting behavior of the compounds may be lost. In that situation, fast decay of the signal strength will be observed. However, there are other factors that may cause the decrease in signal strength. Thus, from the decay pattern, no conclusion may be drawn with great precision. On the other hand, if the contrast agent changes its optical absorbance property after its action as a cancer drug, a different photoacoustic signal may

be produced by illuminating the target with another laser pulse. Comparing these signals with the previous signals, the drug activity of the contrast agents may be determined. The ratio of two signals will give the fraction of drug molecules (here the contrast agent) reacted. From the time difference between two laser pulses, the rate of the reaction of drug molecules could be determined. If a short series of laser pulses are implemented systematically, the progress rate of the drug action will be mapped quantitatively. A schematic diagram is presented below to illustrate this proposal.

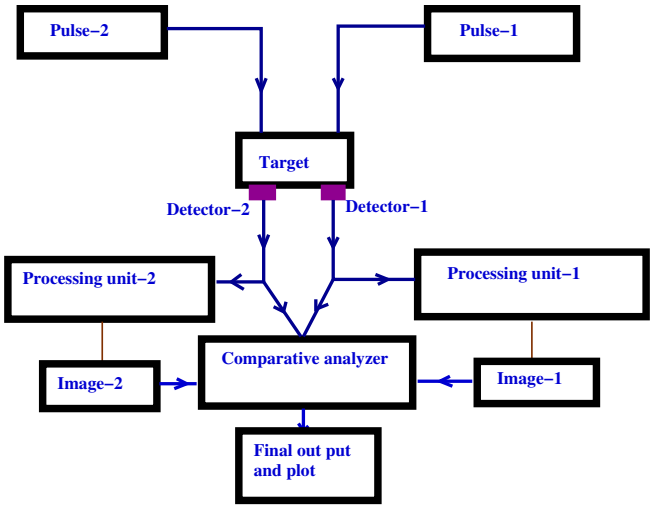

Figure 1: A schematic diagram of proposed TD-PAT

Description and method of calculation

In Figure 2, the target is under the focus of two different laser pulses. Laser pulse-1 corresponds to the frequency of the used contrast agent before its drug action and laser pulse-2 corresponds to the frequency of the contrast agent after its drug action. There are two independent detectors. Here it should be mentioned that when pulse-1 is on, detector-1 will be on and detector-2 will be off. For pulse-2, the reverse will be true. These conditions are required to avoid the mixing of two different signals as both the detectors are capturing signals from the same tissue surface. There should be a significant time gap between the application of two laser pulses. Signals that are recorded at two different detectors are passed

to two independent processors to get independent images produced from two different laser pulses. Both these signals will also be sent to an analyzer where a comparative study will be processed. The analyzer will also collect two images and compare them to give the calculated result.

From the signal ratio, the fraction of drug molecules that have reacted could be calculated in the following way.

Let, x be the fraction of drug molecules that have reacted. So, the amount of unreacted drug molecules is $1-x$. As the strength of the signal is proportional to the concentration of the contrast agents, we get

$$\frac{signal-2}{signal-1} = \frac{x}{1-x} \tag{1}$$

$\frac{signal-2}{signal-1}$ is a number that is available from the designed TD-PAT instrumentation described above. Let the measured value is K. Now, from Eqs. 1 we get

$$\frac{x}{1-x} = K \tag{2}$$

so,

$$x = \frac{K}{1+K} \tag{3}$$

x is the drug activity index. Employing pulse-2 more than once, we will get different values of x. The plot of x vs $time$ will show the progress of the drug activity.

Computational details

To test the efficiency of triruthenium-carbonyl cluster derivatized with glucose-modified bicyclophosphite ligands as an effective TD-PAT contrast agent (PAT contrast agent and cancer drug), the optical absorbance property of this compound and its derivatives with DNA base is studied. For this purpose, geometry optimization of these compounds is carried out with

the help of the Gaussian 09 package.[36] The structure optimizations were carried out without any symmetry restrictions following standard methodology. Becke's three-parameter hybrid exchange functional combined with Lee-Yang-Parr non-local correlation functional, abbreviated as B3LYP, with LanL2DZ basis, is employed for the present computation. It is reported that for the transition metal clusters B3LYP/LanL2DZ produces excellent results compared to the experimental results.[19–22] Time-dependent density functional theory (TD-DFT) calculations were performed to study the optical absorbance of the compounds under test for their electronic excitations.

Results and Discussion

The optical absorbance of different triruthenium carbonyl cluster derivatives due to their electronic excitation is computed and presented in Figure 2. Mono substituted derivative is chosen for this study. It is reported that this compound acts as a cancer drug by stopping cell division through a mechanism where the compound binds to two DNA bases of the opposite strands of DNA when it starts to unfold at the G1/G0 stage of the cell division.[27] Thus, the optical absorbance of the mono substituted metal cluster and its derivatives with DNA base Guanine is computed and reported.

In Figure 2, it is observed that the absorption maximum of the monosubstituted derivative and its mono-substituted guanine derivative are 505 nm and 556 nm respectively. The di-substituted guanine derivative shows two distinct absorption peaks. The first peak, the low energy peak, is at 1085 nm and the second one is at 484 nm. Thus, this compound, triruthenium carbonyl cluster derivatized with glucose-modified bicyclophosphite ligands, is a promising candidate for the TD-PAT contrast agent. For this compound, the frequency of the laser pulse-1 must be around 500 nm and that of pulse-2 should be around 1100 nm. Since the required frequency of two pulses is widely separated, this could be easily used as a contrast agent for TD-PAT. The signal corresponding to an 1100 nm laser pulse is directly

related to the activity of the ruthenium complex as well as the rate of cell division which is related to the spread of cancer cells. It is also observed that there is another significant absorption of the di-substituted guanine derivative at 480 nm. So, the calculation of x should be modified accordingly. From the image obtained from laser pulse-2, the arrested cell division area would be visible.

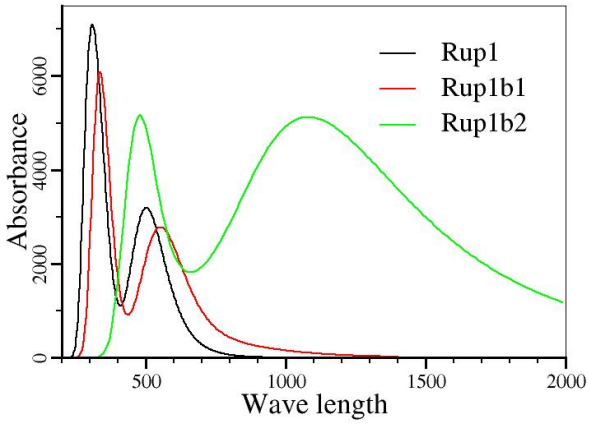

Figure 2: Optical absorbance properties of different cluster derivatives.

Conclusion

From the present study, it could be concluded that it is possible to construct a TD-PAT instrument. The required contrast agent for this technique is obtainable for cancer. The triruthenium-carbonyl cluster derivatized with glucose-modified bicyclophosphite ligand (mono-substituted) is an effective contrast agent to detect the progress of cancer in a living organism. It may also be a good drug for this disease. It is observed that when the cluster binds with a DNA base of a single strand, there is no significant change in its optical absorption property. But, this property changes drastically only when it binds to two DNA bases of two different strands of an unfolding DNA which stop the cell division and the spread of cancer. Thus, the success of the chemotherapy could be noticed spectroscopically if this compound is used for

the treatment. Monitoring of the therapy could also be possible in this case. This technique (TD-PAT) will give more flexibility in the future to resolve clinical and medical complexities.

Acknowledgement

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References

(1) Bell, A. G. ART. XXXIV.–On the production and reproduction of sound by light. *American Journal of Science (1880-1910)* **1880**, *20*, 305.

(2) Tam, A. C. Applications of photoacoustic sensing techniques. *Reviews of Modern Physics* **1986**, *58*, 381.

(3) Wang, L. V. Prospects of photoacoustic tomography. *Medical physics* **2008**, *35*, 5758–5767.

(4) Thakor, N. V. Highlights: Transcranial imaging of functional cerebral hemodynamic changes in single blood vessels. *Journal of Cerebral Blood Flow & Metabolism* **2012**, *32*, 936–937.

(5) Wang, X.; Pang, Y.; Ku, G.; Xie, X.; Stoica, G.; Wang, L. V. Noninvasive laser-induced photoacoustic tomography for structural and functional in vivo imaging of the brain. *Nature biotechnology* **2003**, *21*, 803–806.

(6) Wang, X.; Pang, Y.; Ku, G.; Stoica, G.; Wang, L. V. Three-dimensional laser-induced photoacoustic tomography of mouse brain with the skin and skull intact. *Optics letters* **2003**, *28*, 1739–1741.

(7) Stephens, D. J.; Allan, V. J. Light microscopy techniques for live cell imaging. *science* **2003**, *300*, 82–86.

(8) Jaiswal, J. K.; Goldman, E. R.; Mattoussi, H.; Simon, S. M. Use of quantum dots for live cell imaging. *Nature methods* **2004**, *1*, 73–78.

(9) Tsytsarev, V.; Bernardelli, C.; Maslov, K. I. Living brain optical imaging: technology, methods and applications. *Journal of neuroscience and neuroengineering* **2012**, *1*, 180–192.

(10) Clarke, L.; Velthuizen, R.; Camacho, M.; Heine, J.; Vaidyanathan, M.; Hall, L.; Thatcher, R.; Silbiger, M. MRI segmentation: methods and applications. *Magnetic resonance imaging* **1995**, *13*, 343–368.

(11) Lu, W.; Huang, Q.; Ku, G.; Wen, X.; Zhou, M.; Guzatov, D.; Brecht, P.; Su, R.; Oraevsky, A.; Wang, L. V., et al. Photoacoustic imaging of living mouse brain vasculature using hollow gold nanospheres. *Biomaterials* **2010**, *31*, 2617–2626.

(12) Sarma, A.; Heilbrun, M. E.; Conner, K. E.; Stevens, S. M.; Woller, S. C.; Elliott, C. G. Radiation and chest CT scan examinations: what do we know? *Chest* **2012**, *142*, 750–760.

(13) Lamb, C. R. Ultrasonography of the ureters. *Veterinary clinics of North America: Small animal practice* **1998**, *28*, 823–848.

(14) Kim, T.; Zhang, Q.; Li, J.; Zhang, L.; Jokerst, J. V. A gold/silver hybrid nanoparticle for treatment and photoacoustic imaging of bacterial infection. *ACS nano* **2018**, *12*, 5615–5625.

(15) Chen, Y.-S.; Frey, W.; Kim, S.; Kruizinga, P.; Homan, K.; Emelianov, S. Silica-coated gold nanorods as photoacoustic signal nanoamplifiers. *Nano letters* **2011**, *11*, 348–354.

(16) Firouzi, K.; Stride, E.; Saffari, N. A theoretical investigation of photoacoustic contrast agents. *The Journal of the Acoustical Society of America* **2013**, *133*, 3853–3862.

(17) Hellebust, A.; Richards-Kortum, R. Advances in molecular imaging: targeted optical contrast agents for cancer diagnostics. *Nanomedicine* **2012**, *7*, 429–445.

(18) Zha, Z.; Deng, Z.; Li, Y.; Li, C.; Wang, J.; Wang, S.; Qu, E.; Dai, Z. Biocompatible polypyrrole nanoparticles as a novel organic photoacoustic contrast agent for deep tissue imaging. *Nanoscale* **2013**, *5*, 4462–4467.

(19) Bag, A.; Ghorai, P. K. Computational investigation of the ligand field effect to improve the photoacoustic properties of organometallic carbonyl clusters. *RSC Advances* **2015**, *5*, 31575–31583.

(20) Bag, A.; Ghorai, P. K. Enhancement of biocompatibility and photoacoustic contrast activity of metal clusters. *Journal of Molecular Graphics and Modelling* **2017**, *75*, 220–232.

(21) VoonáKong, K.; KeeáLeong, W., et al. Organometallic carbonyl clusters: a new class of contrast agents for photoacoustic cerebral vascular imaging. *Chemical Communications* **2014**, *50*, 2601–2603.

(22) Bag, A. Application of glucose modified bicyclophosphite derivative of tri-ruthenium carbonyl cluster as advanced photo acoustic contrast agent. *Saudi J. Med. Pharm. Sci* **2015**, *1*, 80–82.

(23) Pillai, M. M.; Karpagam, K.; Begam, R.; Selvakumar, R.; Bhattacharyya, A. Green synthesis of lignin based fluorescent nanocolorants for live cell imaging. *Materials Letters* **2018**, *212*, 78–81.

(24) Kong, J. W.; Lam, Z.; Chan, K. H.; Ganguly, R.; Joey Lee, J.-Y.; Loo, L.-H.; Webster, R. D.; Wong, Z. X.; Leong, W. K. Group VIII Metal Carbonyl Cluster-Boronic Acid Conjugates: Cytotoxicity and Mode of Action Studies. *ACS omega* **2021**, *6*, 29045–29053.

(25) Kong, K. V.; Leong, W. K.; Ng, S. P.; Nguyen, T. H.; Lim, L. H. Osmium carbonyl clusters: a new class of apoptosis inducing agents. *ChemMedChem: Chemistry Enabling Drug Discovery* **2008**, *3*, 1269–1275.

(26) Kong, K. V.; Chew, W.; Lim, L. H. K.; Fan, W. Y.; Leong, W. K. Bioimaging in the mid-infrared using an organometallic carbonyl tag. *Bioconjugate chemistry* **2007**, *18*, 1370–1374.

(27) Nazarov, A. A.; Baquié, M.; Nowak-Sliwinska, P.; Zava, O.; van Beijnum, J. R.; Groessl, M.; Chisholm, D. M.; Ahmadi, Z.; McIndoe, J. S.; Griffioen, A. W., et al. Synthesis and characterization of a new class of anti-angiogenic agents based on ruthenium clusters. *Scientific reports* **2013**, *3*, 1–7.

(28) Jakupec, M. A.; Galanski, M.; Arion, V. B.; Hartinger, C. G.; Keppler, B. K. Antitumour metal compounds: more than theme and variations. *Dalton transactions* **2008**, 183–194.

(29) Clarke, M. J. Ruthenium metallopharmaceuticals. *Coordination Chemistry Reviews* **2002**, *232*, 69–93.

(30) Allardyce, C. S.; Dyson, P. J. Ruthenium in medicine: current clinical uses and future prospects. *Platinum Metals Reviews* **2001**, *45*, 62.

(31) Pongratz, M.; Schluga, P.; Jakupec, M. A.; Arion, V. B.; Hartinger, C. G.; Allmaier, G.; Keppler, B. K. Transferrin binding and transferrin-mediated cellular uptake of the ruthenium coordination compound KP1019, studied by means of AAS, ESI-MS and CD spectroscopy. *Journal of Analytical Atomic Spectrometry* **2004**, *19*, 46–51.

(32) Hartinger, C. G.; Jakupec, M. A.; Zorbas-Seifried, S.; Groessl, M.; Egger, A.; Berger, W.; Zorbas, H.; Dyson, P. J.; Keppler, B. K. KP1019, a new redox-active anticancer agent–Preclinical development and results of a clinical phase I study in tumor patients. *Chemistry & biodiversity* **2008**, *5*, 2140–2155.

(33) Sava, G.; Gagliardi, R.; Bergamo, A.; Alessio, E.; Mestroni, G. Treatment of metastases of solid mouse tumours by NAMI-A: comparison with cisplatin, cyclophosphamide and dacarbazine. *Anticancer Research* **1999**, *19*, 969–972.

(34) Chatterjee, S.; Kundu, S.; Bhattacharyya, A.; Hartinger, C. G.; Dyson, P. J. The ruthenium (II)–arene compound RAPTA-C induces apoptosis in EAC cells through mitochondrial and p53–JNK pathways. *JBIC Journal of Biological Inorganic Chemistry* **2008**, *13*, 1149–1155.

(35) Nowak-Sliwinska, P.; van Beijnum, J. R.; Casini, A.; Nazarov, A. A.; Wagnieres, G.; van den Bergh, H.; Dyson, P. J.; Griffioen, A. W. Organometallic ruthenium (II) arene compounds with antiangiogenic activity. *Journal of medicinal chemistry* **2011**, *54*, 3895–3902.

(36) Frisch, M. J. et al. Gaussian 09 Revision D.01.

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