J Fluoresc (2014) 24:305–311
DOI 10.1007/s10895-013-1331-6

SHORT COMMUNICATION

# Overlap of Doxycycline Fluorescence with that of the Redox-Sensitive Intracellular Reporter roGFP

**Heba Khader · Victor Solodushko · Abu Bakr Al-Mehdi · Jonathon Audia · Brian Fouty**

Received: 20 August 2013 / Accepted: 20 November 2013 / Published online: 28 November 2013


**Abstract** Tetracycline-inducible systems allow for either suppression or induction of transgene expression to facilitate studies of cell physiology. Doxycycline is a preferred inducer for these gene expression systems due to its membrane permeability; however, the heterocyclic structure of doxycycline exhibits fluorogenic properties that can potentially bias measurement of other fluorochromes. Thus the simultaneous use of tetracycline-inducible systems and fluorescent proteins as reporter genes or as intracellular biosensors may lead to potentially confounding results. Herein, using cells which co-express the ratiometric redox sensitive intracellular reporter, roGFP, and a tetracycline-inducible reporter plasmid encoding the reporter gene, mCherry, as a model system, we describe the overlapping intracellular fluorescent signals between doxycycline and commonly used intracellular fluorescent probes. In our cells, the addition of doxycycline to cells caused a dose- and time-dependent increase in cell fluorescence with 405 nm excitation which overlapped with that of the oxidized configuration of roGFP. Incubating cells in concentrations of doxycycline less than 1 μg/mL and removing doxycycline from the media 60 min before performing experiments eliminated fluorescence interference while still maintaining maximal reporter transgene activation.

**Keywords** Doxycycline · roGFP · Tetracycline-inducible system · Fluorescent proteins · Fluorochromes

H. Khader · V. Solodushko · A. B. Al-Mehdi · B. Fouty
Department of Pharmacology and the Center for Lung Biology, University of South Alabama School of Medicine, Mobile, AL 36688, USA

J. Audia
Department of Microbiology and Immunology and the Center for Lung Biology, University of South Alabama School of Medicine, Mobile, AL 36688, USA

B. Fouty
Department of Internal Medicine and Division of Pulmonary and Critical Care Medicine, University of South Alabama, School of Medicine, Mobile, AL 36688, USA

B. Fouty (✉)
Center for Lung Biology, Department of Pharmacology, MSB 3406, University of South Alabama School of Medicine, Mobile, AL 36688, USA
e-mail: bfouty@health.southalabama.edu

## Introduction

Fluorescent proteins are extensively used in molecular biology research as reporter proteins to mark transgene expression or to track the localization and trafficking of proteins of interest for live cell imaging. Fluorescent proteins are also used as intracellular biosensors that can detect changes in pH [1], $Ca^{2+}$ concentration [2], or the oxidative state of the cell [3]. Tetracycline-regulated (Tet-on and Tet-off) systems are commonly used to induce or suppress transgene expression in cultured cells. The tetracycline analogue most commonly used to induce transgene expression in Tet-on systems, doxycycline, has fluorogenic properties due to its heterocyclic structure. Doxycycline is excited at wavelengths between 390 and 425 nm with an emission fluorescence at wavelengths between 520 and 560 nm [4]. The use of both tetracycline-inducible systems and fluorescent proteins in the same cells requires the ability to distinguish the fluorescence of intracellular biosensors from the fluorescence of doxycycline itself.

To examine the utility of combining intracellular redox probes with tetracycline-inducible expression systems, we stably infected rat pulmonary microvascular endothelial cells (PMVEC) with the ratiometric redox-sensitive green fluorescent protein, roGFP-2. roGFP is a biosensor fluorescent protein prepared by the introduction of two redox-sensitive

cysteine residues into green fluorescent protein [3]. The oxidation of these residues results in a conformational change of roGFP that alters its fluorogenic properties. roGFP has two excitation maxima (approximately 400 nm and 490 nm) with an emission peak around 510 nm. Oxidation of roGFP increases the emission with 400 nm excitation and decreases the emission spectrum with 490 nm (or the more commonly used 480 nm) excitation which allows for real-time analysis of the redox state of the cell. Since the excitation/emission spectrum of doxycycline overlaps with that of roGFP, we stably transduced these roGFP-expressing PMVEC with a tetracycline-regulated mCherry reporter gene and determined the effect of increasing doxycycline concentrations on reporter gene expression and intracellular fluorescence. The addition of doxycycline increased cell fluorescence following 405 nm excitation which overlapped with that of the oxidized configuration of roGFP. Removing doxycycline from the media led to a return to baseline fluorescence within 60 min. Incubating cells in concentrations of doxycycline less than 1 μg/mL decreased fluorescence interference while still providing adequate transgene activation.

## Materials and Methods

### Cell Culture

Rat pulmonary microvascular endothelial cells (PMVEC), rat pulmonary artery endothelial cells (PAEC) and rat smooth muscle cells (SMC) were isolated by the Center for Lung Biology cell culture core facility at University of South Alabama as previously described [5].

### Generation of roGFP Retrovirus and Tetracycline-Inducible System and Infection of Rat PMVEC

To generate the roGFP plasmid, roGFP-2 was excised from a custom-made plasmid pMA2704 (gifted by Mikhail Alexeyev) using *Age*I and *Sac*I restriction sites. The roGFP fragment was cloned into a pSIREN-retroQ-ZsGreen (Clontech, Mountain View, CA, USA) retroviral plasmid replacing the ZsGreen gene at *Age*I and *Xho*I sites with the sequential removal of an upstream U6 promoter from the plasmid (*Bg*lII-*Eco*RI fragment deletion). To generate the tetracycline inducible (Tet-on) plasmid, the mCherry coding sequence was excised from the pRSET-B mCherry plasmid (Invitrogen) and cloned into the multiple cloning site of a custom-made tetraycyline-inducible retroviral vector pMA2780 (gifted by Mikhail Alexeyev). The lentiviral vector pFUW-M2rtTA (Addgene, Cambridge, MA, USA, Cat.# 20342) was used to deliver the transactivator into target cells. The transactivator (rtTA)-expressing lentiviruses were propagated in HEK-FT cells by co-transfection of pFUW-M2rtTA, psPAX2 and pMD2.G plasmids (all from Addgene). Media containing rtTA and mCherry viruses were filtered and used to infect rat PMVEC that stably expressed roGFP in a 1:1 proportion. Three days after infection, doxycycline (2 μg/mL) was added to the cell culture media and cells positive for both mCherry and GFP sorted by flow cytometry and expanded.

### Flow Cytometry Measurements

The mean fluorescence intensity of trypsinized viable cells was recorded under AmCyan (405 nm) and FITC (488 nm) channels using the Becton Dickinson Canto II flow cytometer. For mCherry expression, rat PMVEC were incubated with different concentrations of doxycycline for 48 h and analyzed under PE-TexasRed (532 nm) channel using the Becton Dickinson FACSAria II flow cytometer. To account for the background signal, wild type rat PMVEC were analyzed under the same channels and the background signal was subtracted from the rat PMVEC roGFP or rat PMVEC mCherry signal. 10,000 cells were analyzed per experiment and each experiment was repeated 3 times.

### Spectrofluorometry

All emission scans were done using a photon-counting spectrofluorometer (PTI Inc. Lawrenceville, NJ, USA) and a quartz microcuvette under a fixed temperature of 37 °C. Excitation and emission slits were set at ±5 nm bandpass. Doxycycline emission spectra in the range of 430–600 nm were acquired with an excitation wavelength of 400 nm in PBS, cell homogenates, and whole cell suspensions at concentrations ranging from 0 to 200 μg/mL. A 250 μl aliquot of either PBS, cell lysates, or whole cell suspension was added to quartz microcuvettes and emission spectra acquired. After an initial baseline reading, 50 μg/mL of doxycycline and/or 50 mmol/L Ethylenediaminetetraacetic acid (EDTA) were added and the emission scans repeated.

### Statistical Analysis

Data are expressed as mean ± SEM. Results were analyzed using linear regression, paired t-test or repeated measures ANOVA with Dunette's post hoc analysis as appropriate. $P$ value$<0.05$ considered significant.

## Results and Discussion

We first stably expressed the redox-sensitive green fluorescent protein (roGFP) in rat pulmonary microvascular endothelial cells (PMVEC) by retroviral transduction and then confirmed the functionality of roGFP in our system by exposing cells to different concentrations of hydrogen peroxide ($H_2O_2$) for

10 min. As expected [6], $H_2O_2$ caused a dose-dependent increase in fluorescence intensity of the 405 nm signal accompanied by a decrease in the 488 nm signal leading to an increase in the 405/488 ratio consistent with roGFP oxidation (Fig. 1).

We next determined the fluorescence dose–response curve of doxycycline in solution by spectrofluorometry. Figure 2 demonstrates the emission scan of doxycycline over a concentration range from 0 to 200 μg/mL in phosphate buffered saline solution (PBS). Fluorescence above baseline was not detected at concentrations of doxycycline below 5 μg/mL. Since doxycycline's excitation/emission spectrum overlaps with that of the oxidized form of roGFP, we next tested whether doxycycline could alter roGFP fluorescence in cells in the ranges commonly used to induce transgene expression in tetracycline-inducible systems (0.25–2 μg/mL) [7–9]. Rat PMVEC overexpressing roGFP were treated with doxycycline concentrations from 0 to 4 μg/mL. Flow cytometric analysis showed that doxycycline increased the 405 nm signal without affecting the 488 nm signal in a dose-dependent fashion (Fig. 3a). Interestingly, an increase in fluorescence could be detected at doxycycline concentrations as low as

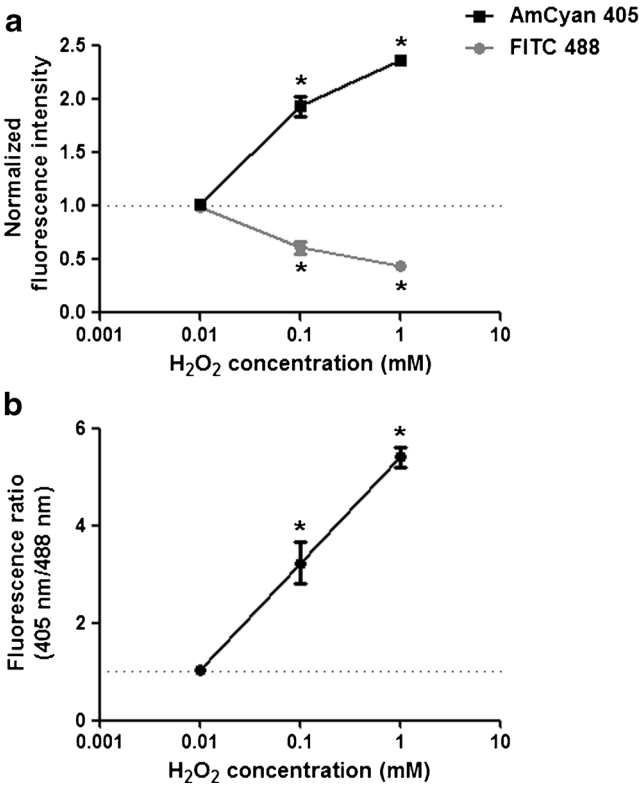


**Fig. 1** Dose response curve of hydrogen peroxide on roGFP oxidation in rat PMVEC. Rat PMVEC expressing roGFP were treated with 0, 0.01, 0.1, or 1 mmol/L of $H_2O_2$ for 10 min, and then analyzed by flow cytometry using AmCyan (405 nm) and FITC (488 nm) channels (**a**). The ratio of the 405 to 488 nm signal, which represents roGFP oxidation, is shown in (**b**). The results shown are mean ± SEM of three independent experiments

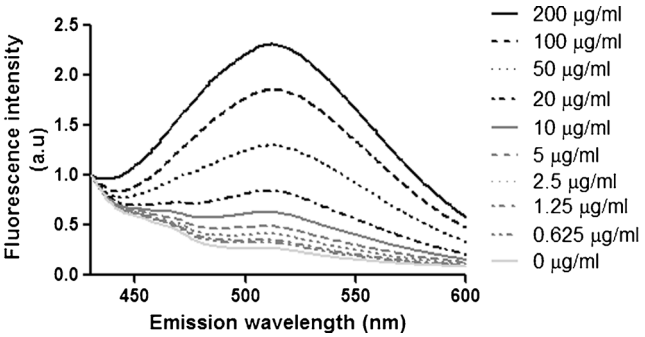


**Fig. 2** Dose–response emission spectrum for doxycycline in PBS when excited at 400 nm as determined by spectrofluorometry (a.u.: arbitrary units)

1 μg/mL, a concentration below the limit of fluorescence detection of doxycycline dissolved in PBS alone. The addition of doxycycline at 2 μg/mL led to a rapid increase in fluorescence intensity with 405 nm wavelength excitation which increased over 30 min (Fig. 3b).

To determine whether the increase in fluorescence signal intensity upon addition of doxycycline to roGFP-overexpressing cells was due to its interaction with roGFP, we incubated wild type (non-roGFP transfected) rat PMVEC with increasing concentrations of doxycycline and determined changes in fluorescence over time. The addition of doxycycline to wild type cells also increased the fluorescence intensity when excited at the 405 nm wavelength in a dose- (Fig. 3c) and time- (Fig. 3d) dependent manner. Furthermore, the extent of the observed increase was similar between wild type and roGFP-overexpressing cells. As with roGFP-expressing cells, the fluorescence intensity of doxycycline-loaded cells excited at the 488 nm wavelength did not change and was similar to that of vehicle-treated (PBS) cells. Together these data suggest that some property of the cells was affecting doxycycline fluorescence rather than the roGFP protein *per se*.

The augmented doxycycline fluorescence upon exposure to cells raised the question as to whether the doxycycline was concentrating within the cell or whether it was interacting with a cellular constituent such as proteins or ions. Thus, we compared doxycycline fluorescent intensity (50 μg/mL) in PBS alone, PBS containing rat PMVEC cell lysates, or PBS containing intact cells. When incubated with cell lysates (obtained by mechanical disruption and centrifugation), doxycycline fluoresced six times more brightly than in PBS alone (Fig. 4a and b). When incubated with whole cells, fluorescence was approximately 20 % greater than with cell lysates. Considering that doxycycline can interact with divalent cations such as calcium and magnesium leading to increased fluorescence [10], we added the chelator ethylenediaminetetraacetic acid (EDTA) after adding doxycycline. The addition of EDTA (50 μmol/L) decreased fluorescence in cell lysates by 45 % and in whole cells by 65 %, but it unexpectedly

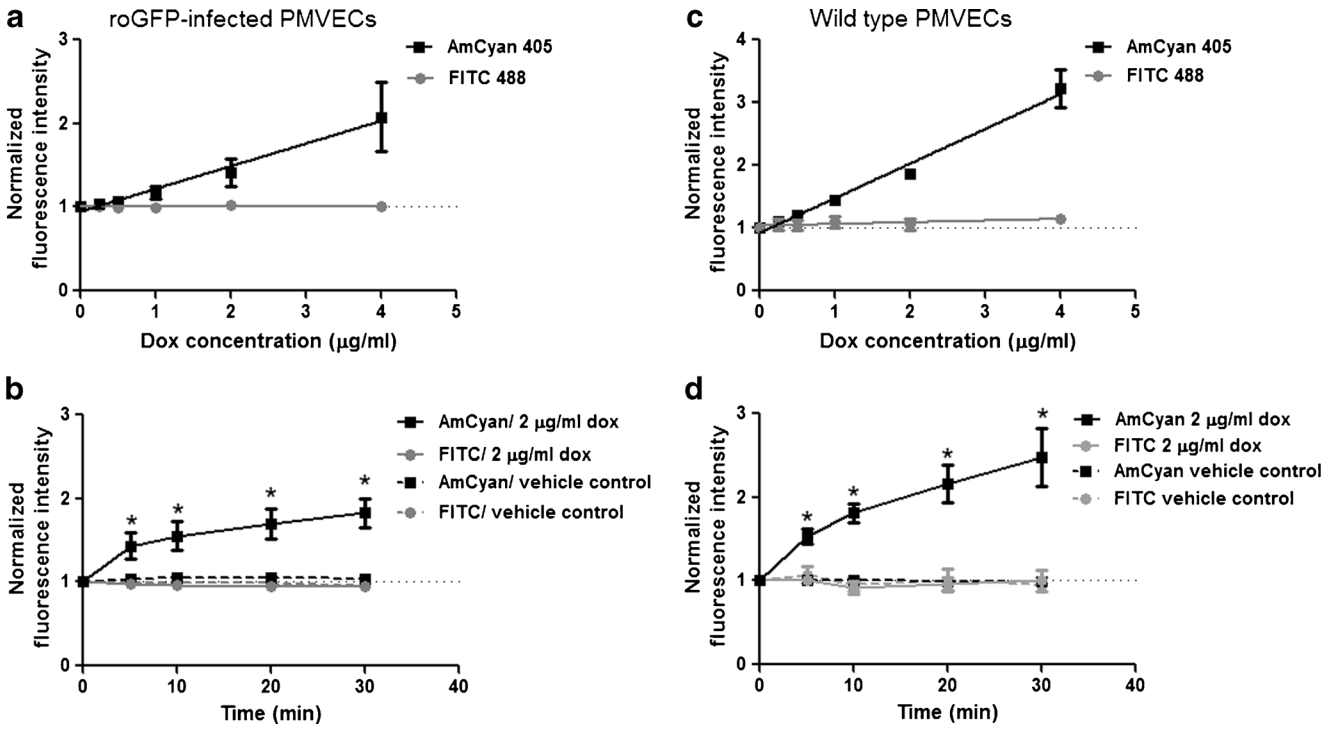


**Fig. 3** Doxycycline increased the intensity of the 405 nm signal in a dose- and time-dependent manner in roGFP-infected and wild type rat PMVEC as determined by flow cytometry. After initial readings (time 0), doxycycline was added to roGFP-expressing rat PMVEC to a final concentration of 0.25, 0.5, 1, 2, or 4 μg/mL and analyzed by flow cytometry using AmCyan (405 nm) and FITC (488 nm) channels. Results were compared to vehicle control (PBS). The mean fluorescence intensity of AmCyan and FITC channels were recorded after 10 min in roGFP-infected (**a**) and wild type (**c**) rat PMVEC. The fluorescence signal of vehicle control- and the doxycycline-treated (2 μg/mL) cells was monitored over 30 min in roGFP-infected (**b**) and wild type (**d**) rat PMVEC. The results shown are mean ± SEM of three independent experiments

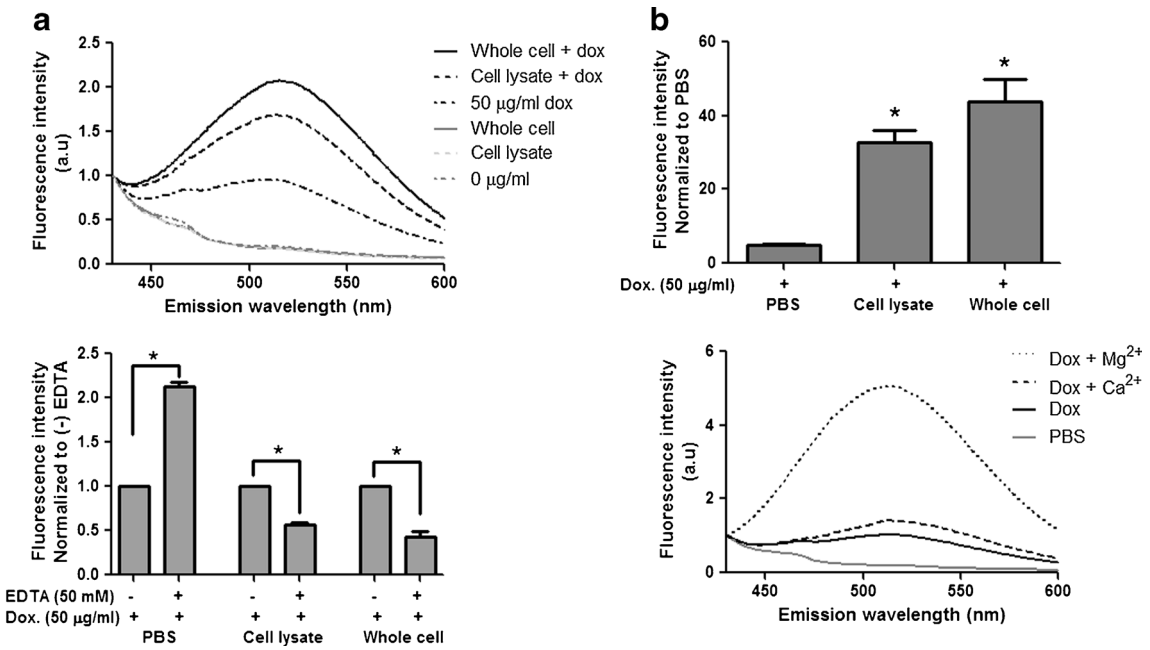


**Fig. 4** Increased doxycycline fluorescence in cell lysates and whole cells relative to PBS due to the presence of divalent cations. **a** Representative emission spectra of doxycycline (50 μg/mL) recorded in PBS, cell lysates, and whole cell suspension when excited at 400 nm as determined by spectrofluorometry. **b** Quantitative analysis of doxycycline fluorescence (50 μg/mL) at 510 nm when excited at 400 nm. **c** Effect of EDTA (50 mmol/L) on doxycycline fluorescence at 510 nm when excited at 400 nm. **d** Effect of calcium chloride and magnesium sulfate on doxycycline fluorescence in PBS at 510 nm when excited at 400 nm. The results shown are mean ± SEM of three independent experiments

increased fluorescence in PBS (Fig. 4c). Closer examination revealed that when excited at 400 nm, EDTA fluoresces at 425 to 440 nm (data not shown), a wavelength that can excite doxycycline. Thus the increase in doxycycline fluorescence in PBS in the presence of EDTA was most likely due to Forster resonance energy transfer (FRET).

In a complementary experiment, we added calcium or magnesium in increasing concentrations to doxycycline in PBS to determine whether it was a direct interaction of divalent cations and doxycycline that caused an increase in fluorescence or whether it required the presence of cell proteins or enzymes. Magnesium added in equimolar concentrations (112.5 μmol/L) increased doxycycline fluorescence in PBS five-fold; the addition of calcium at equimolar concentrations did not increase fluorescence, however (Fig. 4d). A calcium-doxycycline molar ratio of 8:1 was required to augment doxycycline fluorescence to the same degree as an equimolar concentration of magnesium (data not shown). This suggested that a direct interaction of doxycycline with magnesium was the major cause for its increased fluorescence and that magnesium's absence from PBS was the reason for the decreased fluorescence relative to cell lysates and whole cells. Together these experiments suggest that the greater fluorescence of doxycycline observed in whole cells compared with that observed in solution (PBS) was not due to active accumulation within the cell, but rather to its interaction with magnesium. Considering that the intracellular magnesium concentration is estimated to range between 5 and 20 mmol/L [11] and no magnesium is present in PBS, this likely explains the majority of the observed differences.

In our cells, doxycycline concentrations as low as 1 μg/mL led to detectable fluorescence. To determine the doxycycline concentration that was sufficient to activate the Tet-on system in our cells without interfering with roGFP fluorescence, we infected roGFP-expressing rat PMVEC with both the reverse tetracycline transactivator (rtTA) plasmid and a retroviral plasmid encoding the reporter gene mCherry driven by a tetracycline-inducible promoter. Cells were incubated with doxycycline for 48 h and cells positive for mCherry were sorted by flow cytometry and expanded in culture (without doxycycline). These cells were then incubated with different concentrations of doxycycline for 48 h. As shown in Fig. 5, a doxycycline concentration as low as 0.125 μg/mL was sufficient to induce marked mCherry expression in rat PMVEC; at 0.5 μg/mL about 80 % of cells expressed mCherry. Higher concentrations of doxycycline did not further increase the percentage of cells expressing mCherry nor did higher concentrations increase the mean fluorescent intensity of mCherry-positive cells.

In Tet-on systems, doxycycline is usually added for 24 to 48 h to induce transgene expression. To determine if doxycycline's effect on fluorescence persisted over this period of time, rat PMVEC overexpressing roGFP were analyzed under

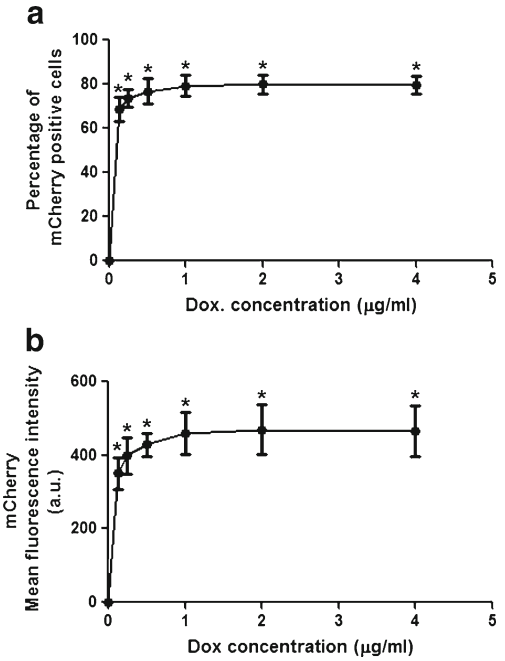


**Fig. 5** Doxycycline can induce mCherry expression in the majority of rat PMVEC at low concentrations. Rat PMVEC infected with a tetracycline-inducible retroviral plasmid encoding mCherry were incubated with different concentrations of doxycycline (0.125, 0.25, 0.5, 1, 2, 4 μg/mL) or vehicle control (PBS) for 48 h. mCherry expression was measured as the percentage of positive cells (**a**) and the mean fluorescence intensity of mCherry positive cells (**b**) using flow cytometry. The results shown are mean ± SEM of three independent experiments

AmCyan and FITC channels after they were treated with doxycycline (2 μg/mL) for 48 h. Similar to its effect during shorter experiments, doxycycline-treated cells demonstrated an increase in signal intensity in cells excited at the 405 nm, but not at the 488 nm wavelength compared to untreated cells (data non shown). We reasoned that if the doxycycline was not sequestered or trapped within the cells, then a brief wash (media change) would facilitate rapid doxycycline efflux out of the cell without affecting reporter transgene levels. Figure 6 shows than even after incubation with doxycycline for 48 h, washing the cells in fresh medium led to a rapid decrease in fluorescence to baseline over 30 to 60 min, an effect that was similar between the four different cell types tested.

When used together in the same cells, intracellular fluorescent probes and tetracycline-inducible systems have the potential to produce confounding results due to the intrinsic fluorescent properties of tetracycline and its derivatives. Doxycycline demonstrates an excitation wavelength between 390 and 425 nm and a major emission peak between 520 and 560 nm [4]. Doxycycline's spectrum has some overlap with that of wild-type GFP which has two absorbance/excitation peaks, one at 395 nm and the other at 470–480 nm, with a major emission peak at 504 nm [12]. Doxycycline's fluorescence spectrum does not overlap with most mutated forms of GFP currently in use as reporter genes, however, which have

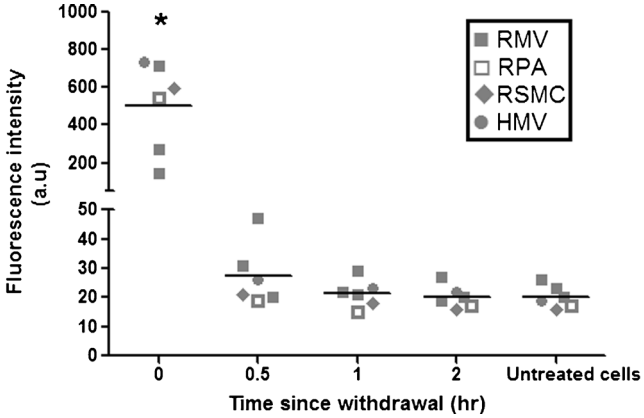


**Fig. 6** Doxycycline's fluorescence decays rapidly after its removal from the culture media. Rat PMVEC, rat PAEC, human PMVEC and rat PASMC were incubated with doxycycline (2 μg/mL) for 48 h. After this incubation period, cells were harvested in trypsin with or without doxycycline and then analyzed by flow cytometry under AmCyan channel (405 nm) over 2 h. The results shown are the mean of three independent experiments for rat PMVEC and one experiment for the other cell types

the major excitation peak around 488 nm [12,13]. This allows doxycycline to be used with most tetracycline-inducible systems that use GFP mutants such as EGFP or GFPS65T as reporter genes. The modifications required to generate the ratiometric redox-sensitive roGFP, however, led to fluorescence overlap between doxycycline and the oxidized form of roGFP, both of which excite at the 405 nm wavelength and emit between 510 and 560 nm.

Fluorochromes other than roGFP also fluoresce within these wavelengths (Table 1). The use of tetracycline-inducible systems with these or other fluorochromes that have excitation/emission spectra that overlap with doxycycline can lead to false positives when sorting with flow cytometry or confusing and uninterruptable results during transgene activation. In our studies, this led to an apparent increase in intracellular oxidation due to fluorescent overlap at the 405/510 nm excitation/emission wavelengths. The presence of doxycycline may also interfere with FRET studies leading to potentially confounding results if an inducible gene is tagged with a fluorophore that overlaps with doxycycline's excitation/emission spectrum. In our studies, EDTA did exactly this. In an attempt to control for stimulation of doxycycline fluorescence by cations, we added EDTA to quench the background fluorescence. While EDTA led to a significant reduction in fluorescence in cell lysates and whole cells, it unexpectedly caused an increase in fluorescence in PBS alone. Closer examination of this phenomenon revealed that when EDTA is excited at 400 nm, it emits at 430–445 nm which is within the excitation range of doxycycline. Thus the increase in doxycycline fluorescence in PBS in the presence of EDTA was most likely due to FRET.

**Table 1** Fluorochromes with excitation/emission spectrum that overlap with doxycycline's spectrum

| Fluorochrome | Excitation (nm) | Emission (nm) |
|---|---|---|
| GFPuv | 385 | 508 |
| T-Sapphire | 399 | 511 |
| Pacific Orange | 403 | 551 |
| Pacific Green | 411 | 500 |
| Lucifer yellow | 425 | 528 |

In solution, doxycycline demonstrated a dose–response fluorescence up to the highest concentration tested, 200 μg/mL, but showed little fluorescence below 5 μg/mL. Yet when tested in cells, doxycycline increased fluorescence at 1 μg/mL. The highly lipophilic nature of doxycycline facilitates its rapid uptake by cells. The increase in doxycycline-mediated fluorescence over time suggested that doxycycline was accumulating in rat PMVEC against a concentration gradient or, alternatively, that it was interacting with intracellular proteins that increased its fluorescence in a time-dependent fashion. While tetracyclines are known to accumulate in both Gram-negative and Gram-positive bacteria [14–16], studies on tetracycline and its derivatives indicate that they do not accumulate in mammalian cells [17], one reason these drugs are relatively non-toxic to eukaryotic cells.

Our results comparing doxycycline fluorescence in PBS, cell lysates, and whole cells demonstrated that rather than accumulating in cells, doxycycline was interacting with magnesium to increase its fluorescence. The addition of EDTA which chelates divalent cations, particularly magnesium and calcium, halved doxycycline's fluorescence in cell lysates and whole cells whereas the addition of equimolar magnesium to doxycycline in PBS increased its fluorescence close to that of cell lysates. This suggested that doxycycline's interaction with magnesium, which concentrates within the cell [18], was an important cause for the increased fluorescence. Chelating magnesium with EDTA almost completely reversed the doxycycline-mediated fluorescence in cell lysates and whole cells (when the FRET effect of EDTA was accounted for).

Most tetracycline derivatives such as minocycline, chlortetracycline, and demeclocycline have similar excitation/emission spectrums to that of doxycycline [19] and therefore changing to a different analogue to regulate the Tet-on/Tet-off system will not offer relief from this problem. Our results suggest two complementary solutions to facilitate the use of tetracycline-inducible systems and intracellular fluorescent probes in the same cells, however. First, very low concentrations of doxycycline appear to adequately activate the tetracycline-regulated systems in cultured rat PMVEC, yet have minimal impact on overall fluorescence. This concentration needs to be directly determined in the cells of interest, however, since extrapolation of doxycycline fluorescence concentration curves obtained in solution do not correlate with those obtained in cells. Second, because removing doxycycline

from the media led to a rapid decline in fluorescence in all four cell types we tested, waiting at least 60 min after the removal of doxycycline before performing FACS or other fluorescent studies will decrease the fluorescent overlap.

**Acknowledgment** This work was supported, in part, by an American Heart Association Grant-in-Aid (AHA-271032) from the Southeastern region.

**Disclosures/Conflict of interest statement** The authors declare no conflict of interest.

## References

1. Llopis J, McCaffery JM, Miyawaki A, Farquhar MG, Tsien RY (1998) Measurement of cytosolic, mitochondrial, and Golgi pH in single living cells with green fluorescent proteins. Proc Natl Acad Sci U S A 95(12):6803–6808
2. Nakai J, Ohkura M, Imoto K (2001) A high signal-to-noise Ca(2+) probe composed of a single green fluorescent protein. Nat Biotechnol 19(2):137–141
3. Hanson GT, Aggeler R, Oglesbee D, Cannon M, Capaldi RA, Tsien RY, Remington SJ (2004) Investigating mitochondrial redox potential with redox-sensitive green fluorescent protein indicators. J Biol Chem 279(13):13044–13053
4. Pautke C, Vogt S, Tischer T, Wexel G, Deppe H, Milz S, Schieker M, Kolk A (2005) Polychrome labeling of bone with seven different fluorochromes: enhancing fluorochrome discrimination by spectral image analysis. Bone 37(4):441–445
5. King J, Hamil T, Creighton J, Wu S, Bhat P, McDonald F, Stevens T (2004) Structural and functional characteristics of lung macro- and microvascular endothelial cell phenotypes. Microvasc Res 67(2): 139–151
6. Dooley CT, Dore TM, Hanson GT, Jackson WC, Remington SJ, Tsien RY (2004) Imaging dynamic redox changes in mammalian cells with green fluorescent protein indicators. J Biol Chem 279(21): 22284–22293
7. Produit-Zengaffinen N, Davis-Lameloise N, Perreten H, Becard D, Gjinovci A, Keller PA, Wollheim CB, Herrera P, Muzzin P, Assimacopoulos-Jeannet F (2007) Increasing uncoupling protein-2 in pancreatic beta cells does not alter glucose-induced insulin secretion but decreases production of reactive oxygen species. Diabetologia 50(1):84–93
8. Lee CF, Qiao M, Schroder K, Zhao Q, Asmis R (2010) Nox4 is a novel inducible source of reactive oxygen species in monocytes and macrophages and mediates oxidized low density lipoprotein-induced macrophage death. Circ Res 106(9):1489–1497
9. Schweiger D, Furstenberger G, Krieg P (2007) Inducible expression of 15-lipoxygenase-2 and 8-lipoxygenase inhibits cell growth via common signaling pathways. J Lipid Res 48(3):553–564
10. Schneider S, Schmitt MO, Brehm G, Reiher M, Matousek P, Towrie M (2003) Fluorescence kinetics of aqueous solutions of tetracycline and its complexes with Mg2+ and Ca2+. Photochem Photobiol Sci 2(11):1107–1117
11. Jahnen-Dechent W, Ketteler M (2012) Magnesium basics. CKJ: Clin Kidney J 5(suppl 1):i3–i14
12. Patterson GH, Knobel SM, Sharif WD, Kain SR, Piston DW (1997) Use of the green fluorescent protein and its mutants in quantitative fluorescence microscopy. Biophys J 73(5):2782–2790
13. Gossen M, Freundlieb S, Bender G, Muller G, Hillen W, Bujard H (1995) Transcriptional activation by tetracyclines in mammalian cells. Science 268(5218):1766–1769
14. Chopra I, Hawkey PM, Hinton M (1992) Tetracyclines, molecular and clinical aspects. J Antimicrob Chemother 29(3):245–277
15. Chopra I, Roberts M (2001) Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. Microbiol Mol Biol Rev 65(2):232–260, second page, table of contents
16. Nikaido H, Thanassi DG (1993) Penetration of lipophilic agents with multiple protonation sites into bacterial cells: tetracyclines and fluoroquinolones as examples. Antimicrob Agents Chemother 37(7):1393–1399
17. Franklin TJ (1966) Mode of action of the tetracyclines. Biochemical studies of antimicrobial drugs. Sixteenth Symposium of the Society for General Microbiology. Cambridge University Press, Cambridge
18. Fouty RA (1978) Liquid protein diet magnesium dificiency and cardiac arrest. JAMA 240(24):2632–2633
19. Pautke C, Vogt S, Kreutzer K, Haczek C, Wexel G, Kolk A, Imhoff AB, Zitzelsberger H, Milz S, Tischer T (2010) Characterization of eight different tetracyclines: advances in fluorescence bone labeling. J Anat 217(1):76–82