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International Journal of Current Research
Vol. 6, Issue, 12, pp.10588-10592, December, 2014

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

CONSPICUOUS BURSTS OF PHOTON EMISSIONS IN MALIGNANT CELL CULTURES FOLLOWING INJECTIONS OF MORPHINE: IMPLICATIONS FOR CANCER TREATMENT

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ARTICLE INFO

Article History:
Received 26th September, 2014
Received in revised form
10th October, 2014
Accepted 25th November, 2014
Published online 27th December, 2014

Key words:

Photon Emissions, Morphine, Metastases, Cell Proliferation, Naloxone, Melanoma Cells.

ABSTRACT

Cultured mouse melanoma cells were removed from incubation to room temperature and injected with either 1 μM of morphine or its mu-receptor antagonist naloxone, or, not treated. Photon emissions were counted measured by a photomultiplier unit for 24 hr. Cells injected with morphine exhibited conspicuous bursts of photon emissions whose amplitudes were as much as 1000 times greater than naloxone-injected or non-treated comparison cells with durations of about 10 s for about one hour. The peaks of the photon bursts in cells injected with morphine were approximately 10^{-11} W·m^{-2} and could involve the synchronized release of energy from all or most of the million cells in the population. The average photon emissions for these cells remained 6 fold greater for 24 hrs. If the multiple spikes and maintained elevation of photon emissions from the morphine-injected but not naloxone-injected or non-treated cells comprise a form of inter-cell communication this phenomenon may partially explain the propensity for morphine treatment to elicit proliferation and metastases in some malignant environments.

INTRODUCTION

Morphine and its derivatives have been considered the most efficacious treatment for the management of pain associated with cancer and its treatment for human patients. Although the responses of opiate receptors within the brain to clinical dosages of morphine facilitate the diminishment of the report of painful experiences, these receptors or their correlates are present in many other cells external to the brain. Opioid receptors are found within neoplastic tissue. Simon and Tracey (1986) found that injections of morphine could encourage metastases of malignant tissue. There is a plethora of literature concerning the molecular "machinery" of cancer cell proliferation. However, the multiple processes by which cells, including malignant forms, "communicate" or exchange intercellular information that may *initiate* intra- and inter-cell processes are still being explored. Within the last few decades there has been a rejuvenation of interest in the mitogenetic radiation of photons from cells that had been pursued vigorously during the 1930s (Gurwtisch, 1988). It is now clear that both normal and malignant cells display copious photon emissions that are easily discernable with contemporary photomultiplier devices. The median of radiant flux density is about 10^{-12} W·m^{-2} (Dotta *et al*., 2011; Kobayashi *et al*., 1999). The basic unit of energy is in the order of about 10^{-20} J per s per cell (Persinger, 2010).

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Initially these photon emissions were considered artifacts of metabolism. Measurements by Dotta *et al*. (2014) who validated the Cosic model for Molecular Resonance Recognition demonstrated that specific frequency bands of photons across the visible range increased or decreased as predicted when specific types of excitatory or inhibitory compounds were added to the cell cultures. The results indicated that ultraweak biophoton transmission from cells could also behave as a form of initial intercell communication (Fels, 2009; Popp, 1988; Tilbury and Quickenden, 1988; Trushin, 2004). Although the powerful and energy-demanding molecular pathways within the cell would clearly be responsible for the changes in physical manifestation, they could be initiated by very weak energies, behaving as a type of "lock and key".

Several researchers have measured photon emissions from cells continuously maintained within incubation (37° C). These conditions often required the application of incident energy to elicit a photon response (Takeda *et al*., 1998; van Wijk and van Aken, 1992). We (Dotta *et al*., 2011) recently found that a variety of normal (non-malignant) and malignant (human and mouse) cells when removed from incubation and placed at room temperature display marked increases in photons for several hours. Our results indicate that the adaptation to this altered temperature is associated with a clear increase in radiant flux density. For mouse melanoma cells, that are particularly malignant and divide quickly (22 hrs), the photon emissions for

a million cells can approach the equivalent of 10^{-20} J per second per cell. This quantity of energy is associated with the resting membrane potential as well as that required to sequester a nucleotide to RNA or a ligand to a receptor.

Injections of morphine have been reported to increase the probability of metastases (Ishikawa *et al*., 1993, Sergeeva *et al*., 1993). Although there are reports that morphine inhibits tumor growth in breast (Farooqui *et al*., 2007) and colon tissues (Harimaya *et al*., 2002), there are also measurements that morphine modulates angiogenesis which is important for primary tumour growth (Gupta *et al*., 2002), invasiveness, and the development of metastases for lung cancer and neuroblastomas. If this relationship is causal and not correlational, and photon emissions from cells exhibit functional significance (Dotta, Saroka and Persinger, 2012; Isojima *et al*., 1995; Scott *et al*., 1991) instead of metabolic artifacts, then significant and conspicuous photon emissions should occur reliably from malignant cells when exposed to clinical levels of morphine. On the other hand exposure to comparable concentrations of morphine antagonists, such as naloxone, should not produce these effects. Here we present what we think is the first evidence that morphine elicits powerful bursts of photon emissions from cancer cells *in vitro*.

MATERIALS AND METHODS

For a total of 9 experiments plates of B16-BL6 cells were cultured to 95% confluence of about 1 million cells. The plates of cells were removed from the normal incubator environment (37° C, 5% CO_2) and injected with 50 μL of 1 μM of morphine sulfate or with 1 μM of naloxone, a morphine μ-receptor antagonist. This reference was considered more appropriate than physiological saline because it accommodated the injection procedure and the concentration of a similar chemical structure that is sequestered to mu-receptors but does not stimulate them. Plates of cells that were not injected with morphine received a new media to maintain handling consistency.

After the administration of the morphine, naloxone or no drug the plates were placed over the aperture of a photomultiplier unit in another room where the ambient temperature was 22° C. The plate and the photomutiplier units were contained with a black box that was covered with multiple layers of black terry cloth towels housed within a darkened basement in order to minimize ambient photons. The procedure was similar to that reported earlier (Dotta *et al*., 2014). Photon counts were measured by a Model DM0090C digital multiplier unit from SENS-TECH, LTD. According to manufacturer's specifications the sensitivity for photons included the range of 280 to 850 nm with a peak between 390 and 420 nm. Photon counts were sampled once every 2.5 s for a total of 24 hours by the equipment software by a laptop computer. The software automatically calculated the photon incidence to numbers of photons per second.

For calculation of energies we assumed the central peak for the PMT to be 500 nm which is equivalent to ~$4.73 \cdot 10^{-19}$ J. When divided by the time in 1 s units the result is Watts. When divided by area of the aperture (~10^{-4} m^2), the equivalent energy per count would be ~$4.7 \cdot 10^{-15}$ $W \cdot m^{-2}$. To accommodate dark counts, that is the background features of the instrument, the counts for the non-treated dishes were subtracted from the counts from the morphine- and naloxone- treated dishes of cells.

RESULTS

The numbers of photons per second for the first 1.7 hours after application of the morphine are shown in Figure 1. The results of two separate trials (experiments) are shown to illustrate the consistency of the effect. These spike patterns were rarely observed after 2 hours, that is, for the subsequent 22 hours when the experiment was completed. This pattern was similar in all triplicates. The duration of the spikes was about 5 to 10 s. The energy equivalents for the larger spikes would be about 10^{-11} $W \cdot m^{-2}$. These spikes were never observed for the cells treated with naloxone or that remained as non-treated controls.

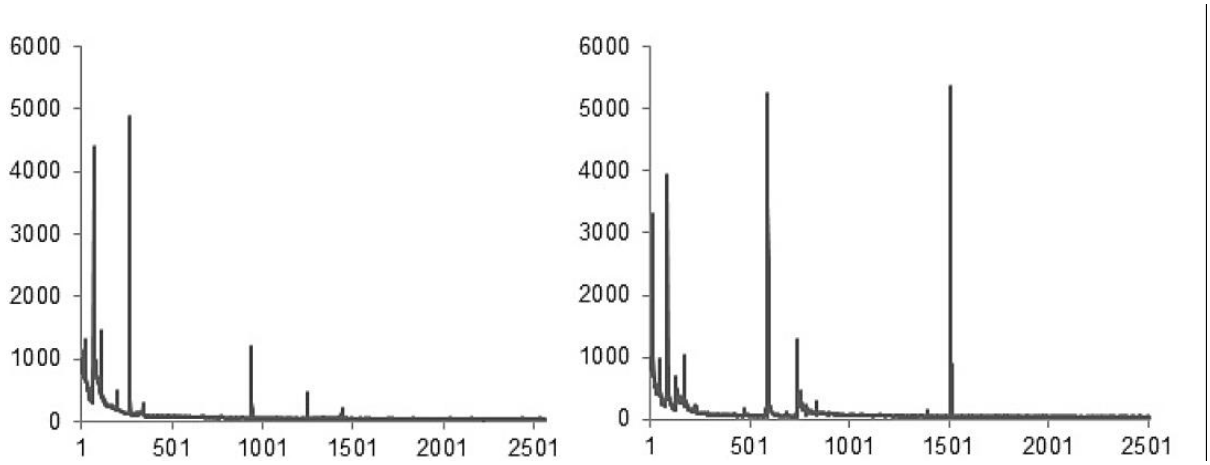

Figure 1. Numbers of photons per second from two separate experiments after morphine was injected into a plate containing 1 million mouse melanoma cells and placed over the aperture of a photomultiplier tube. The duration of the peaks was between 5 to 10 s. The numbers on the x-axis refer to sequential 2.5 s intervals (maximum is 1.7 hrs) after injection of the morphine. Naloxone or non-treated cells never displayed these spikes

The deviations of photon counts of the morphine and naloxone-treated cells shown in Figure 2 were based upon the subtraction of the photon counts per 10 min from the average (mean) value for the entire 24 hr period. The conspicuous increase in photon emissions from the morphine treated cells compared to the naloxone and control cells was equivalent to about 21 photons per s and 4 photons per s, respectively, on average for the first 1 hr after treatment.

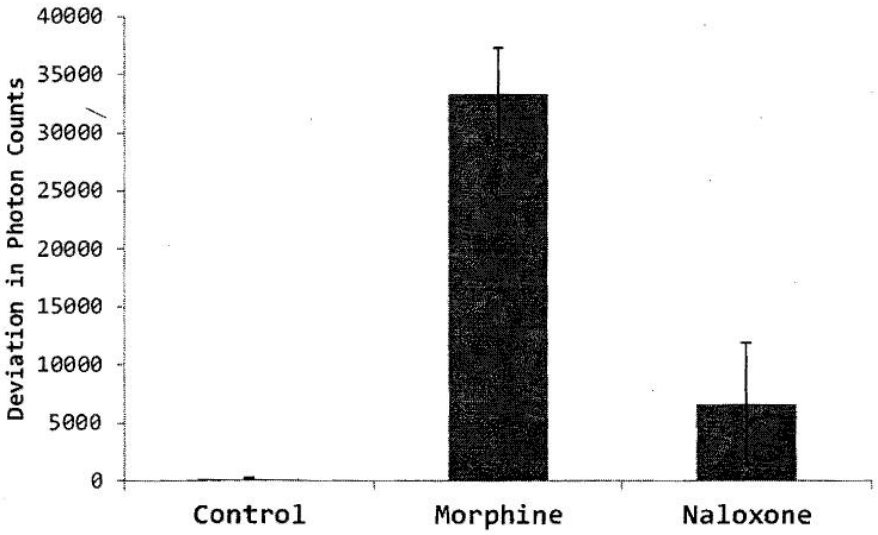

Figure 2. Means and standard deviations for the deviations in photon counts from cells after control (no-treatment) and morphine or naloxone injections

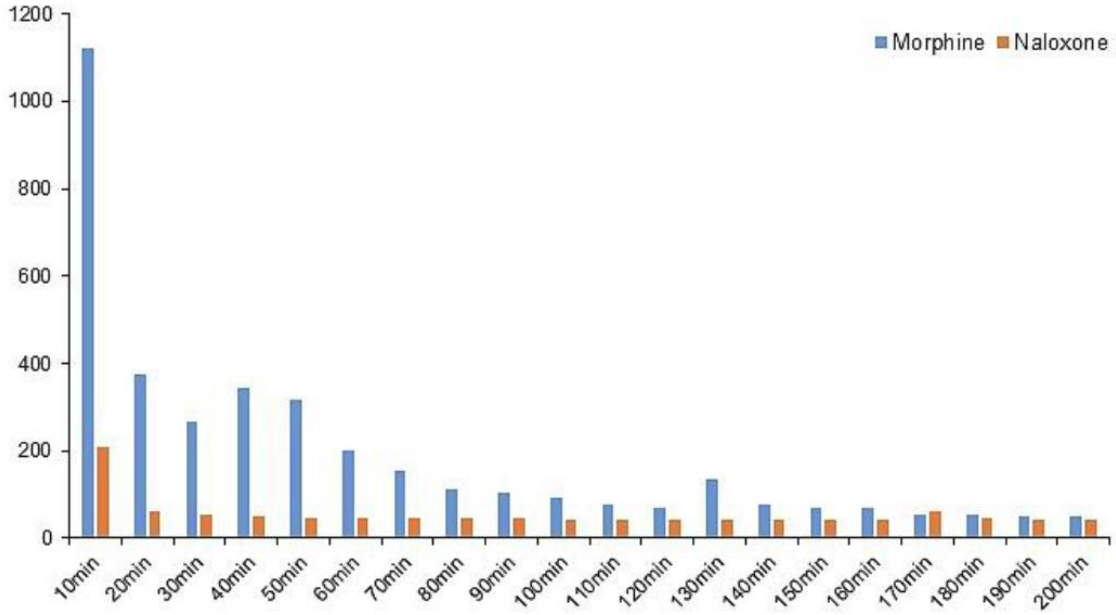

Figure 3. Mean of three trials for photon counts per 10 min increment from plates of cells after they had been injected with either morphine or its antagonist naloxone

To demonstrate the temporal dependence of the photon emission, the average numbers of photons per sec for successive 10 min increments after the application of the morphine or the naloxone for the next 200 min are shown in Figure 3. During the first 10 min after the application of the morphine but not the naloxone, the photon radiant flux density was equivalent to 10^{-12} $W \cdot m^{-2}$. The power density diminished to one-third to one-fourth that power density for the subsequent 50 min. After that time the values were within the range of the cells to which naloxone was applied for the remaining time.

DISCUSSION

The results of these experiments clearly demonstrated that the injection of only 50 µL of 1 µM of morphine into 1 million cells (2.5 cc) resulted in reliable, conspicuous increases in photon emission. This was not observed from cells to which naloxone in a comparable concentration was applied. They did not differ appreciably from non-treated control or reference cells.

All of the cells had been removed from incubation and placed at room temperature over the aperture of a PMT for 24 hr.

Typically these cells emit copious photons, even without treatment. The present results demonstrated that the addition of morphine in a volume that is about 1% of the total volume occupied by the cells produced even more conspicuous photon emissions. On average it was the equivalent of $\sim 10^{-12}$ $W \cdot m^{-2}$ over the first hour above that of the non-treated melanoma cells. On the other hand injection of a comparable volume of naloxone produced minor increases. The average increase in radiant flux density masked the potentially significant "information" that could be considered essential for inter-cell communication. As shown in Figure 1, the largest powerful spikes equivalent to $\sim 10^{-11}$ $W \cdot m^{-2}$ were recorded primarily during the first 30 min after the introduction of morphine into the milieu. The duration of these spikes was between 5 and 10 s. Because we were measuring populations of approximately 1 million melanoma cells these spikes could be considered "population potentials" in a manner analogous to the population potentials measured from neurons in hippocampal slices during monitoring of long-term potential (LTP).

For such spikes to occur we predict that photon emissions of large numbers of melanoma cells would have been coherent or coordinated within the same time. In this instance the actual power over the cell cross sectional area would be 10^{-15} $J \cdot s^{-1}$ (W). If the duration of the burst was about 10 s, then the energy would be 10^{-14} J or the equivalence of 10^{-20} J from each of the approximately 1 million cells. Such coherence, if melanoma cells behave as do glial cell syncytiums, should require some type of inter-cell communication. This indicates that all cells within the population could be potentially affected by the photon burst. In addition a burst with this power density could be discerned potentially by populations of cells several cm in distance. A packet of energy within the range of 10^{-20} J (Persinger, 2010) would be sufficient to affect the addition of a base nucleotide to a RNA ribbon or DNA sequence or the addition or deletion of an amino acid during protein synthesis. This could be sufficient to alter the specificity of the resulting chemistry. In this manner the single 5 to 10 s photon burst distributed over a large population of cells could be a form of communication between cells that could homogenize their chemistry towards the characteristics of the population. Because these cells were malignant this direction would be anticipated. However if the cells were not-malignant, the reconstitution of normalcy might be expected.

The 10^{-20} J magnitude of an increment of energy is within the range associated with the second shell of hydrogen and the movement of protons through the hydronium ion complex of water. Previous calculations have shown that a shift of only 10 nm, effectively the width of plasma cell membrane, of a photon within the visible wavelength such as 470 nm (the peak detection of our PMT) involves the energy equivalent of 10^{-20} J. That the membrane is a primary source of photon emissions, particularly for melanoma cells, was shown experimentally by Dotta *et al*. (2014). The application of concepts of "excess correlation" or entanglement (Persinger and Koren, 2013) whereby "simultaneous" changes in photon properties occur in locations separated by non-traditional distances to cellular activity has rarely been considered. Dotta and Persinger (2010) found that synchronized chemiluminescent reactions separated by either 10 m or 3 km that shared the same circularly rotating magnetic fields that exhibited changing angular velocities exhibited clear evidence of such excess correlations. During these conditions the photon emission from the "entangled" loci displayed photon emissions that were twice the magnitude of that emitted from either site if they did not share these magnetic fields.

The power density of the photon emissions from the hydrogen peroxide and sodium hypochlorite reactions was $\sim 10^{-10}$ $W \cdot m^{-2}$ or only an order of magnitude higher than the total energy released by the most intense spikes measured in the present study in the population of cells injected with morphine. It may be relevant that single cells, because of passive lateral diffusion of molecules in the plasma cell membrane, may exhibit a "membrane magnetic moment" that exhibits the capacity to emit photons when the approximate magnetic field pattern and intensity are applied across a population of cells (Dotta *et al*., 2014b).

Conclusion

Photons are emitted from cancer cells, particularly melanoma cells, when removed from incubation temperature and maintained at room temperature. A single injection of morphine, but not its mu-receptor blocker naloxone, resulted in bursts of photon emissions whose radiant flux densities and durations would be sufficient to influence the increments of energy that determine the order of base nucleotides or amino acid sequences. The quantification strongly suggests that the majority of the cells in the aggregate participate to produce this transient but powerful photon burst. These bursts may be a form of intercell communication that homogenize the chemical activity of cells that respond to the *pattern* of photon energies.

Acknowledgement

We thank Dr. W. E. Bosarge, Jr., CEO Capital Technologies, Inc. for his support of research that emphasizes conceptually different and innovative technologies.

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