International Journal of Innovation Scientific Research and Review
Vol. 05, Issue, 05, pp.4506-4509, May 2023
Available online at http://www.journalijisr.com
SJIF Impact Factor 2023: 6.599

ISSN: 2582-6131

Communication Article

DR JOHN HOLT'S UHF RESONANCE – SENSITIVITY FOR CANCER RADIOTHERAPY AN HYPOTHESIS (Number 3)

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Received 06th March 2023; Accepted 07th April 2023; Published online 31st May 2023

ABSTRACT

Dr John Holt (Radiotherapist, Western Australia) trialed radiowave UHF 434 MHz to treat cancer, claiming that, if it were administered in the 30 minutes prior to radiotherapy (RT), that it increased the cancers' sensitivities to RT greatly. With no satisfactory explanation for this and with the retirement of Dr Holt, it has been neglected. A recent hypothesis suggested that the UHF generated NAD+ and this fueled the deacetylase SIRTUIN2 (SIRT2) which moved into the cell nuclei and perturbed a number of potential steps involving acetylase reactions. The hypothesis suggests that the activated SIRT2 disturbed the cells' DNA damage checkpoint mechanisms, increasing the sensitivity to RT. **Conclusion:** Recent advances may support Dr John Holt's claims that UHF can sensitize cancers to radiotherapy.

Index terms: Cancer, UHF, Microwave, Radiotherapy, checkpoint, sensitivity, SIRT2, PARP1, NAT10, MORC2.

INTRODUCTION

Dr John Holt, Radiologist of Perth, Western Australia, started treating cancer patients with UHF 434 MHz, after preliminary trials in 1974. By 1978 he, and his associate, published a paper (Nelson & Holt 1978) demonstrating that the addition of UHF to radiotherapy (RT) improved the RT results, with the 3 year survival using the combined treatments 54%, against the survival of those with standard RT, 19% (groups n = 52 each). Then, and subsequently, Holt was unable to provide acceptable hypotheses to explain such results, including the "resonance" phenomenon he discovered later (Traill 2022a). He did provide hypotheses, (generally considered to be implausible) but he, and everyone else (critics included), would have been quite unable to explain the observations involving the intracellular events, given the low level of scientific discovery and understanding then, as compared to now. The last few years have seen scientific discoveries that could make hypotheses (at least) possible.

Previous studies (Traill, 2022a, 2022b) were of hypotheses attempting to explain the "resonance" phenomenon described by Holt and some of the cellular changes that may be attributed to it, (rather than to heat effects). In doing so, the potential production, stimulation and effects of the deacetylase SIRT2 were noted, and have been examined here. What has not been examined to date is the claimed increased sensitivity that UHF 434 MHz may contribute to RT. This has been claimed by Holt since 1978, but has been considered inexplicable, effectively "sorcery."

By 1995, a substantial number of patients with cancer (n=329), selected for having measurable cancer deposits, was assembled and analysed, and their survivals over 2 years were documented. Having considerable selection, the study cannot be considered a scientific trial, but it provides interesting and plausible graphs which show features which seem worthy of close study when seeking an hypothesis to explain the claimed increased sensitivity to RT.

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CANCER UHF SENSITIVITY VERSUS PATIENT MORTALITY

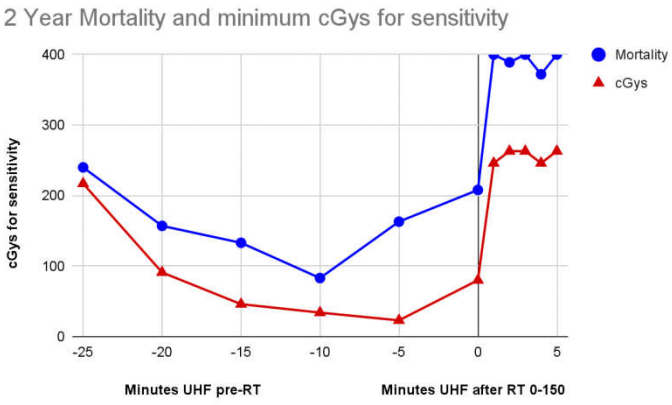

Figure 1. (Adapted from Holt 1995a, 2000b) "329 patients with objectively assessable, proven cancer, previously untreated with RT, UHF or cytotoxics, 1976-1987. RT 150-180 cGy, daily to tolerance." Sensitivity values per cohort (mean? plotted) showed wide spreads: e.g. @ -25 min. 297 cGy, @ -10 min. 217 cGy, @ 0 min. 366 cGy, @ 30-150 min ~137 cGy.
The 329 patients were allocated into 11 cohorts, each with variable patient numbers.
Mortality was recorded after 2 years, expressed as a fraction and related to the 400 scale of the Y axis.
The graphs are inverted here to show features more clearly, and to highlight the abrupt cessation of any new sensitivity benefit once the RT starts. The smaller the cGys for "sensitivity," the more sensitive is a patient's cancer because the smaller value can achieve the target level of damage with less RT dose.
Despite the uncertainties, acknowledged bias and variables, the graph lines show reasonably plausible forms, justifying general comments towards an hypothesis.

(Earlier, Holt presented a preliminary graph with smaller patient numbers [Holt 1983c]. The points were of means.)

(Note that the horizontal scale changes close to the right edge to 0 – 150, and the "5" should be ignored.)
(In comparing the clinical treatment times and responses with in vitro studies, remember that the latter usually lack relevant chaperones, molecules that hold the involved molecules in optimum shapes and forms and which then function better.)

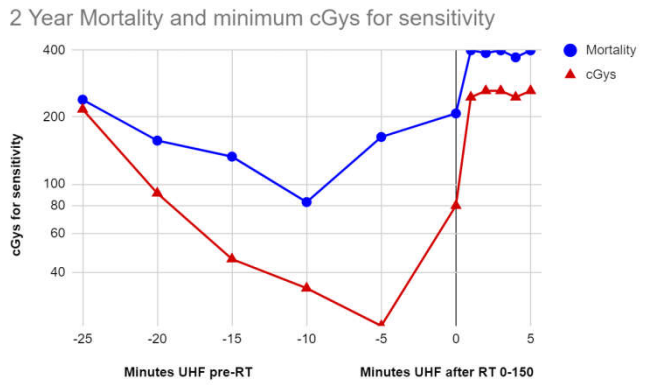

Figure 2. Figure 1 again, with the vertical scale now logarithmic. The cGys (sensitivity) graph is now closer to a straight line, incorporating (perhaps) two similar slopes, each of ~10 minutes. The improvement in mortality seems more related to the early -25 to -15+ than to the -10 to 0 minutes before the RT, when the sensitivity seems greatest.

With the UHF completed ~5 minutes prior to RT, the cGys at 0 min. may indicate that biochemical reactions are not complete; with increasing time between the treatments, the sensitivity deteriorates, as though there are one or more labile reactants losing potency with time, independent of RT exposure.

THERE ARE 3 MAIN FEATURES:

- **Sensitivity Induction with UHF completed before Radiotherapy (RT) start** (-25 to 0 minute). The effectiveness seems to start at about -20 min. and increases, peaking at about -5 min. After this, there is some loss until 0 min. Thereafter, there is, seemingly, a complete block on any UHF benefit. The ability of the RT to stop the UHF from having any benefit from 0 min. onwards (to 150 min.) must have **significance** (see below)..
  Without RT, UHF must create a state that make subsequent RT more effective, yet RF blocks further induction towards sensitivity.
- **The Transition UHF/RT.** One may wonder how easily it was to move patients from the UHF machine to the RT machine ! This interval may be reflected in the wide dispersion of readings at 0 min.
- **UHF following the start of RT.** Seemingly, at no time after 0 min. did the applied UHF after the RT commencement confer any appreciable induction benefit to patients. Whatever biochemical changes created by the prior UHF were, still lingered and conferred increased sensitivity to the RT treatment.

EXPLANATION ATTEMPT – AN HYPOTHESIS:

- **A previous hypothesis** (Traill 2022a) suggests that the UHF energy is conducted through the cancer cells mainly along the microtubules; being associated with, and attached to the α-tubulin molecules (Siegel *et al.,* 2018) are the enzymes NQO1 and (SIRT2).
- With UHF power travelling along the microtubules, the attached molecules (SIRT2 & NQO1 etc.) may be detached &/or increase activity.
- The "resonance" phenomenon explained earlier (Traill 2022a) is expected to draw energy (as electrons) from NADH, producing NAD+, a fuel for SIRT2 and PARP1, the former probably mainly in the cytoplasm, especially around cell division.
- **SIRT2,** associates with, and deacetylates perinuclear α-tubulin (Nielsen *et al.,* 2021), and is an NAD^+-dependant histone deacetylase (interacting overall with >200 other proteins e.g. HOXA10, Bae *et al.,* 2004) in both the cytoplasm and the nucleus. It also deacetylases a number of other cytoplasmic components, in particular, the participants of the Anaphase-promoting Complex/Cyclosome complex, and others involved in mitosis, such as Aurora A (Kim *et al.,* 2011). However, these components are not described as having any particular sensitivity to Ionizing Radiation (IR)/RT, so attention moved to examining intranuclear components.
- SIRT2 is dephosphorylated at -S25 and can move into the nucleus. When appropriately stimulated (as by an infection), and probably activated by the bacterial protein InlB, the cell receptor Met and downstream phosphatidylinositol 3-kinase (PI3K)/AKT signaling, the SIRT2, dephosphorylated by the myristoylated phosphatases PPM1A & PPM1B (Chida *et al.,* 2013, Pereira *et al.,* 2018) can be actively imported through the nuclear membrane to the chromatin of the nucleus by several importin proteins (being regulated by its C-terminus), with one or more of importin subunits alpha-1 (KPNA2), Importin 7 (IPO7), Transportin 1 (TNPO1) and Importin (IPO9) required.
- It translocates from the cytosol to the chromatin of the host at the transcription start sites of a subset of genes that are then repressed, relevant to the involved infection (Eskandarian *et al.,* 2013). (This bacterial-stimulated account may not be applicable in cancers.) Its level in the cytoplasm is low early in the cell cycles (G_1-S) but increases through pre-mitosis (G_2) to mitosis (M), at times when the nuclear membrane is breaking-down, allowing the cytosol to soak the chromosomes and the microtubular spindle. Those contents that had been enclosed by the nuclear membrane, would be able to dissipate, and with stress and an oxidative medium (as probably pertains with UHF) could be deacetylated by SIRT2 and ubiquinated (Zhang *et al.,* 2021). Before the nuclear membrane disintegrates, SIRT2 can enter the nucleus, the rate of nuclear export can match or exceed the rate of nuclear import (North & Verdin 2007) so it is normally found in quite small amounts there. However, if the "back-doorman" CRM1, guarding the nuclear exit pores, is inhibited/damaged by the chemical Leptomycin, or ionizing radiation (as with Radiotherapy, RT/IR; Long *et al.,* 2022), the intra-nuclear concentration of SIRT2 could build up appreciably, indicating that, normally, there is a brisk shuttling cycle through the nucleus (Inoue 2007) whilst the nuclear membrane lasts.
- **SIRT2 nuclear activity** can be towards the histone lysine 16 (H4K16) &/or H3K18 and gene repression following a bacterial stimulus (Eldridge *et al.,* 2020) but a more general disturbance may be created by deacetylating histone H3K56 in the H3 core domain (Vempati *et al.,* 2010). In the nucleus it may induce G_2 arrest of the cell cycle with persistence of Cyclin B/cdc2 following stress (Inoue *et al.,* 2007).

(Subsequently, SIRT2 may be ubiquitinated Dryden *et al.,* 2003 and Liu *et al.,* 2020 :

SIRT2 + CDC14B SIRT2 <===> HRD1
(HMG-CoA) reductase
↓ ↓
degradation 1 26S Proteasome Ubiquination &
Ubiquination degradation)

In the current context, the SIRT2 activity is potentially directed to the deacetylation of MORC2K767Ac (see below).

IONIZING RADIATION (As by RT)

- **The effects of ionizing radiation** (IR, Radiotherapy/RT) have been meticulously studied by scientists recently (Liu et al., 2000, Liu et al., 2020, Zhang et al., 2020, Zhang et al., 2022, Zheng *et al.,* 2022) and the following summary comes largely from them :
- **Key factors** that influence genes and histones here are (Liu *et al.,* 2020):
  a) PARP1 Some 40% of PARP1 is in the nucleolus (Rancourt & Satoh 2009).
  b) SIRT2 – a deacetylase, primarily cytoplasmic, but also nuclear (see earlier),
  c) MORC2 - oncogenic chromatin-remodeling enzyme
  d) NAT10 – an acetyltransferase in the nucleolus when without stress/IR, then è MORC2K767Ac(etyl)
  e) GSN5 – an histone acetyltransferase
  e) Histone H3 phosphorylation at threonine 11 (=H3T11P)
  f) CDK1 and Cyclin B1 - contributing to DNA damage-induced G2 checkpoint activation/halt.
  g) Eg5 – A kinesin-related motor needed for mitosis, transports related to microtubules

A. Intra-nucleolar:

a) **Poly (ADP-ribose) polymerase 1 (PARP1)** is at a relatively high concentration within the **nucleolus**, and seems to be the IR-sensitive "trigger" to respond to, or initiate events, in the IR/RT-induced reaction cascade of interest (being the induced sensitivity to IR/RT).
b) **Activated PARP1** reacts with, and activates (by PARylation), the intra-nucleolar enzyme N-acetyl transferase 10 (NAT10), which can then move out of the nucleolus and into the nucleoplasm. (Liu *et al.,* 2022)

B. Intra-nuclear (surrounding the nucleolus/nucleoli):

a) In the nucleoplasm, PAR-activated NAT10 combines-with and activates the enzyme MORC family CW-type zinc finger 2 (MORC2) by acetylation of MORC2 Lysine 767 (= MORC2K767Ac) (Liu *et al.,* 2022).
b) The acetylation step to MORC2K767Ac can be countered by the deacetylase SIRT2 arriving from the cytoplasm (see above). **This seems a vulnerable point in the cascade** – the point at which **SIRT2 may be able to stop the cascade progression, leading to the IR failing to induce mitosis arrest.**
c) NAT10 binds, acetylates Eg5 K771 and co-localizes with Eg5 in the centrosome during mitosis, and stabilizes it. **This may be another vulnerable point where SIRT2 may, by deacetylation, also cause a failure to induce mitosis arrest.**
d) With DNA damage, but **without** a SIRT2- induced block, MORC2 proceeds to dephosphorylate Histone 3 at Threonine 11 (= H3T11P; Shimada *et al.,* 2007). This, correlating with reduced binding of histone acetyltransferase GCN5 at *cyclin B_1* and *cdk1* promoters, **reduces H3-K9 acetylation.** A reduction of H3K9Ac at the promoters of *cdk* and *Cyclin B_1* results in reductions of Checkpoint factors CDK and Cyclin B_1 .[Also, other factors such as CDC20, Aurora-A & Aurora-B may be vulnerable to SIRT2 (Kim *et al.,* 2011).] These could produce a G_2/M failure and a disturbed response to the damaging agent (e.g. IR/RT) allowing mitotic catastrophe (= sensitivity to IR/RT).

Accordingly, there are a number of sites in the damage response cascade where an elevated level of SIRT2 might break cascade continuity, blunting the Checkpoint halt in the progression and permit the ongoing survival and passage of cells with defective/lethal genomes, onward to mitotic catastrophe.

UHF is Applied (pre RT)

a) Following-on from the hypothesis (Traill, 2022a), excess NAD+ reaching the nucleoplasm may fuel :

i. **PARP1**. This may increase sensitivity for IR detection when RT is delivered subsequently, and this may be countered by automodification (Pascal 2018).
ii. **SIRT2** in the nucleus (normally fluctuating between very low levels to mild cyclically). This may block or neutralize the acetylation stages involving the constitutional PARP1-NAT10 Acè MORC2K767 steps that would exist in most cancers prior to RT. This would halt progression through to the histone-involved steps and Checkpoint 2 failure and gives rise to RT sensitivity, because a pause for chromosomal and gene repair is lost. **THEN** -

RADIOTHERAPY (RT/IR) APPLIED

With Radiotherapy applied and DNA strands being damaged, PARP1 is diverted to DNA repair, leaving little to activate/PARylate NAT10, attenuating the **induction** of further sensitivity changes. Some may acetylate MORC2K767 but this step might have some moderation due to residual SIRT2 from the UHF; but the residual SIRT2 is more likely swamped, and the cascade can proceed. **Residual** effects at the histone end of the cascade may have sensitizing effects persisting into the RT treatment.

However, the **RT may damage** the nuclear membrane's CRM1 (Inoue 2007), allowing SIRT2 & NAD^+ to maintain some ongoing presence in the nucleus and attenuate the pathway that leads from MORC2 to phosphorylating Histone 3 at Threonine 11 (= H3T11P), then to Checkpoint 2 failure and RT sensitivity.

SUMMARY of STEPS (Hypothesis)

i. UHF induction: SIRT2+NAD^+ ➔ a) Nucleus/nucleolus and ubiquinates **PARP1** (Zhang *et al.,* 2022)
b) **MORC2**K767Ac is blocked
c) **NAT10 binds, acetylate Eg5** K771 is blocked
d) Promoters for ***Cdh1* & *CyclinB1*** are blocked
➔ RT: Checkpoint cascade blocked ==> **RT Sensitive.**

ii. UHF stop, then RT started ➔ Abrupt transition; Sensitivity indu: ➔ PARP1 activated for **DNA repair** now, **diverted away from NAT10Ac & MORC2**

CONCLUSION

Some 45 years ago, Radiotherapist Dr John Holt published work based upon clinic cancer patients. He claimed that UHF 434 MHz Radio wave, when applied to patients in the 30 minutes before radiotherapy, sensitized the cancers, allowing lower radiotherapy doses, fewer side effects and better results.

This essay has attempted to present an explanatory hypothesis, based upon modern scientific information, outlining a possible biochemical explanation of the phenomenon. Perhaps this may stimulate interest in Holt's work and discoveries and, from lessons learnt, may lead to improvements in patient treatments and management. Much more research is welcoming.

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