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Research Article

DR JOHN HOLT'S CANCER TRIAL – HYPOGLYCAEMIA, L-GLUCOSE & UHF AN HYPOTHESIS (Number 4)

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ABSTRACT

Dr John Holt (Radiotherapist, Western Australia) trialed radiowave UHF 434 MHz after insulin-induced hypoglycaemia, L-Glucose and then [L-Glucose + D-Glucose] infusions, to treat late breast cancer. He claimed very gratifying results. Given the unique form of treatment, details of the cases and an analysis of the possible biochemical effects are presented as an hypothesis here, referring to more recent scientific information. Perhaps aspects of Holt's work may have application in new ways today. **Conclusion:** Recent advances may support Dr John Holt's claims that UHF and glucose modulations may have value in cancer treatment.

Keywords: Breast Cancer, UHF, Microwave, Hypoglycaemia, L-Glucose, D-Glucose, NHE, MCT4, NQO1.

INTRODUCTION

L-Glucose. In his efforts to boost the effectiveness of the UHF 434 MHz resonance effect in cancer treatment, Holt (Holt, 1979 and 1980) tried administering intravenous L-Glucose prior to UHF applications. He had considered that, by depleting D-glucose supply to cancers, their energy would be reduced and the cancer cells would succumb. Replacing D-Glucose with L-Glucose might achieve this, because only a few soil bacteria seem capable of metabolizing L-Glucose (Shimizu *et al.,* 2012). The D-glucose access to the cytoplasm is mainly through (i) facilitated diffusion (Glucose Transporters; GLUTs) and (ii) Na^+/sugar co-transport (SGLT), (Ono *et al.,* 2020). With the development of fluorescent tracers attached to D-glucose (2-NBDG) and L-glucose (2-NBDLG) there seemed prospects for a clearer understanding of sugar handling (Kao *et al.,* 2021). When used for *in vitro* studies, there seemed the possibility that there could be a non-GLUT/non-SGLT yet unidentified mechanism participating in the uptake of the fluorescent tracer-labeled L-glucose in 2 dissimilar tumour cells, summarized by the possibility that some cells express at least two pathways for taking-up the fluorescent analogues of glucose – (i) a possibly non-transporter-mediated pathway that enables the uptake of both fluorescently-labeled L-glucose and D-glucose analogues in a non-stereo-selective manner, and (ii) a conventional glucose transporter such as a GLUT-mediated pathway, that carries the D-form predominantly (Ogawa *et al.,* 2021). These *in vitro* studies used cells from a small number of origins, with the implication that the observed L-glucose uptake was infrequently found.

Because Hamilton *et al.,* (2021) were concerned about the effects that the large tracer attached to the sugar molecules might have, their further testing indicated that pharmaceutical inhibition of GLUT1, nor genetic manipulation of it changed the binding/uptake of the [D-glucose+tracer], calling into question the value of the tracer for assessing glucose transport. But, there were no research findings that seemed to conflict with Holt's earlier observations (to be examined).

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CLINICAL APPLICATION

By themselves, the L-Glucose infusions appeared to be disappointing. When intravenous L-glucose (4 g) was administered intravenously to cancer patients, the established temperature rise from the subsequent incident UHF, seemed to be ~halved, with the rate of rise to rebound upon administration of 25 g D-glucose:

Testing 5 late-stage cancers/sarcoma, of colon, lung, breast x2 and sarcoma, directing UHF such that the incident and "reflected" (from tumour) power to each patient was maximum, (after 15 minutes for each reading), the mean values for temperature rise (+/- standard deviation; Holt, 1980) from (i) starting temperature, 0.7°C/2min/100Kg (0.12); (ii) then following L-glucose, 1.7°C/2min/100Kg(0.09); (iii) then following D-glucose, 4.1°C/2min/100Kg(0.28); with all patients showing similar trends (i.e. not a rare response). From these results, access by L-glucose to the cytoplasm of many human cancers seems likely (i.e. not via rare mutations). How this occurs is not clear. But once there, it is unlikely to find a binding site (structural or enzymatic) of importance, meaning that further involvement may result from a) the ability to act as a **reducing agent** or b) **hypertonic** effects.

The reducing role might resemble the Fehling's reaction :

$2Cu(OH)_2$ + reducing sugar $\rightarrow$ $2Cu_2O$ + Aldonic acid
(See Figure 1)

The clinical effects of L-Glucose seemed to: (i) quench the (presumed) UHF resonance at 434 MHz then, (ii) The subsequent addition of intravenous D-glucose restores (presumed) full heat/resonance (with reflected emitted power) producing (claimed) augmented clinical benefit. (The final clinical outcomes/survivals of these patients are not presented.)

Despite small patient numbers and some uncertainties, these results would seem consistent with L-glucose gaining access into the cytoplasm of the cancer cell masses, process uncertain. Following on from the above, when intravenous [L-glucose + UHF] (seemingly ineffective) was followed by an infusion of [D-Glucose +UHF], the

results are reported to be extremely gratifying. Outcomes are presented (Table 1) from only 2 patients (female breast cancers with ulcerating chest wall lesions that healed and biopsies of the areas were clear). Why Dr Holt did not pursue this variation in protocol is not known, but it would seem to deserve more attention and analysis.

Table 1. The patients

Feature	Patient 1	Patient 2
Weights (x Photographs)	~60 kg (?)	~60 kg (?)
Cancer (advanced)	Breast (chest wall, fungating)	Breast (chest wall, fungating)
Insulin dose (for hypoglycaemia)	480 i.u. intravenously	720 i.u. intravenously
Initial L-Glucose i.v. infusion	250 mg/kg	250 mg/kg
UHF 434 MHz (First course)	**3 alternate days**	**3 alternate days**
Insulin dose (for hypoglycaemia)	480 i.u. intravenously	720 i.u. intravenously
Second i.v. infusion, D- & L-Glucose	50 ml 50% D-Glucose &75 mg/kg L-Glucose	50 ml 50% D-Glucose & 75 mg/kg L-Glucose
UHF 434 MHz (Second course)	**3 consecutive days**	**3 consecutive days**
Clinical Outcome (claimed)	Clinically clear (photograph) Skin biopsies x5 "no cancer seen"	Ulceration remained (photograph) Skin biopsies x3 "no cancer seen"

CONDITIONS:

Temperature: Holt (1979) studied the temperature changes occurring within his patients when irradiated with up to (potentially) 2,400 Watt UHF of 434 MHz. His findings were :

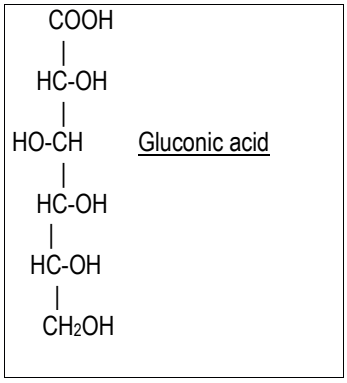
a) The whole body temperature (= rectal/"core") expressed as the **rise** in degree ^{o}C per kg per minute:
Cancer patients (average) 0.0038 , range 0.0018 to 0.0051
Controls (no known cancer) 0.0014, range 0.0011 to 0.0017
b) Tumour temperature. Cancers under radiowave irradiation are hotter than the surrounding more normal tissues. Holt recorded that such preferential temperature rises in 41 patients were between 0.8 ^{o}C and 4.0 ^{o}C (average rise 1.9 ^{o}C) at 15 minutes.

The relevant enzyme NQO1 has a narrow *in vitro* active enzyme range with heat, but retains ~98% activity at 40 ^{o}C (Zaboli, 2000) Relating these to the two patients who participated in the trial with L-Glucose: both patients would appear to have body weights very approximately 60 kg. If their whole body temperatures at the start were 37 ^{o}C, their whole body temperatures could rise by ~2.28 ^{o}C in 10 minutes UHF exposure, and their cancers could have differential temperatures of ~1.9 ^{o}C.

Discussion: Interest in the access of the D- & L- glucoses into cancer cells has arisen with the development of fluorescent tags for the sugars (Ono *et al.,* 2020; Ogawa T, Sasaki A, Ono K *et al.,* 2021. But access is still possible, but by an uncertain means.

Reduction: The L-Glucose may add reducing capacity, and the Gluconic acid produced may cause acidification. These changes will be in an environment in the cytoplasm with 3 important components – NADH/NAD^+, NADPH/$NADP^+$ and NQO1.

Figure 1



If so, L-Glucose could not only reduce, but acidify (in a reaction that may be more vigorous with heat). The release of Hydrogen ions (H^+; protons) from the acid into the cytosol can be countered by two main mechanisms:

PROTON EXCHANGERS:

a) **The Sodium Hydrogen Exchange molecules (NHE)**. These are Adenosine triphosphate (ATP)-dependent and less effective in oxidizing conditions (Brown *et al.,* 1991, Affar *et al.,* 2002, Hu *et al.,* 1998) and are primarily responsible for the regulation of intracellular pH (Rich *et al.,* 2000). With the patients in induced hypoglycaemic states, with the tumour glycolysis pathways depressed and ATP production low, this exchanger would be close to inactive – the cytosolic pH would fall low, and be little affected by this pathway.

b) **The Monocarboxylate transporter 4 (MCT4).** This also can expel Lactate and protons (H^+) and raise the cytosolic pH (Gerlinger, 2012; using renal cell carcinoma cells rich with MCTs). This has been studied in relation to suppressing malignant cell growth (Man *et al.,* 2022) and the Warburg effect (Gerlinger *et al.,* 2012). Increasing the pH activates key metabolic "gatekeeper" enzymes for the glycolytic and pentose phosphate pathways such as Hexokinase (HK), one of four isoforms of Pyruvate Kinase (PKM2) and Glucose 6 Phosphate Dehydrogenase (G6PD) providing increased NAD(P)H, with stimulation of cell proliferation and, with UHF, (potential) energy.

[The intranuclear PARPs, when stimulated by chromosomal damage, are supplied by NAD^+ and release H^+. Whilst the (cytoplasmic) Tankyrases have PARP components, can stimulate tumour growth by glycolysis when over-expressed (Yang *et al.,* 2019), and their inhibition stimulates tumour proliferation, there is no clear indication that they respond to specific damage in a similar way to chromosomal damage (Zamudio-Martinez *et al.,* 2021). Their roles are uncertain here.]

First Infusion – The L-Glucose Effects:

a) **Acidification** of the cytosol by L-Glucose (see above) and,

b) **Tonicity**. The L-Glucose ("4 g in 30-50 mL water"; [whereas 1g per 20 mL water is considered isotonic]) infusion may make the extracellular tissues, and then the cytosols of cancer cells hypertonic. Assuming this, and given that hypertonic saline can stimulate the NHE exchanger and raise the pH of lymphocytes in normal subjects

(Düsing *et al.,* 1994) then, when the NHE exchangers are supplied with ATP, the NHE exchanger may be activated and raise the pH (see later).

c) **The enzyme NAD(P)H:quinone oxidoreductase 1** (**NQO1**, EC 1.6.99.2 = DT-Diaphorase) is a homodimeric, flavin-dependent, two-electron reductase. In the reducing environment the molecule is "closed" with enzyme activity and attachments to other molecules inhibited (Ross & Siegel, 2017; Siegel *et al.,* 2018; Ross & Siegel, 2021). With little/no glycolysis, the supply of NAD(P)H would be minimal for electron donation to the UHF resonance. NQO1 has a narrow pH range for catalytic activity. If the *in vitro* readings can be related to the *in vivo* applications, the optimum pH for activity is ~10.5, the activity falling to ~80% at pH ~10, then ~50% at pH ~9.5 (Zaboli *et al.,* 2020). Any acidification created by the reducing reaction of L-Glucose would be expected to add additional suppression to the NQO1 enzyme.

Second Infusion -D-Glucose + L-Glucose and UHF

Features:

- **Glycolysis,** (important in cancer cells Warburg,1956), needing D-Glucose. Glycolysis is re-awakened , producing ATP, and is now available to activate the NHE Exchangers, raising the pH of the cytosol, providing a much less favourable environment for cancer.
- **NQO1** – This is considered to be readily induced (Ross & Siegel, 2017). The change in expression of NQO1, (a target of Nrf2, measured using qPCR), showed ~x3.5 fold increase upon 100 µM esculetin (antioxidizing) treatment at 8 h, then~x5 at 12 h (Arora, *et al.,* 2016). The enzyme activity (*in vitro*) has an optimum at 37°C and is ~98% at 40°C, but falls to ~80% at 1 h (unless production is sustained), (Zaboli *et al.,* 2020). Presumably, there will be some carry-over to the next day (an unknown extent). The spectral patterns from Holt's patient with Human Immuno-deficiency Virus (HIV; Traill, 2022) a) July 28 – b) August 7 shows a greatly enlarged "resonance" below, at and above the incident frequency consistent with an hypothesized augmentation derived from increased NQO1 production which was stimulated by the UHF/oxidizing infusion events. Response to x-irradiation is rapid, (Boothman *et al.,* 1993). With two cell lines, human melanoma and non-foetal human fibroblasts; following x-irradiation (500cGy), the increase of the specific mRNA for DT-diaphorase (=NQO1) starts at ~1 h, peaks after ~4 h, there attaining from x 33-66 of base value (depending upon the base value), then having an irregular, ~linear fall to the base value at ~16 h. Heat-shock had no appreciable stimulation; UV radiation stimulation was strong, but phorbol 12-myristate 13-acetate, was mild.
- **NAD(P)H** (reducing action) a) Both NADH and NADPH (Ma *et al.,* 1990) equally induce a change in the structure of NQO1 (Siegel 2018) such that the molecule undergoes a shape change, from a "closed" form under reducing (predominant; *Enz-FADH*$_2$) conditions, to an "open" form when under oxidizing conditions (*Enz-FAD)*. When "open" the catalytic site (near the FAD component) is exposed. Then the molecule can react more actively with others in the cytoplasm, including the microtubule molecules. The change to the reducing environment closes access. b) Provides electrons for the UHF resonance (Traill, 2021). c) Raises the reducing power in the cytosol, leaving less demand on L-Glucose to reduce and to produce acid, thereby decreasing acidification.
- **Tonicity.** Continued infusion of L-Glucose maintains the tonicity of the cytosol, stimulating the NHE Exchangers and so creates/sustains an higher pH.
- **UHF** is applied for resonance, adding an oxidizing environment by drawing electrons to generate the emitted UHF patterns..

With such reactivation, there are potentials for overshooting (e.g. L-Glucose supplying hypertonicity and stimulating an ongoing pH rise) – to end up with a better than expected cancer-killing result.

Table 2. Summary of treatment stages

Treatment stage (Holt's)	Biochemical stage &interpretation
1) Fasting (?), then insulin-induced hypoglycaemia	Glycolysis is minimized, so that ATP production is minimal NAD(P)H production is minimal $NAD(P)^+$:NAD(P)H falls below Redox threshold for NQO1 (*Enz-FADH*$_2$)
Infusion of L-glucose i.v.	L-Glucose reducing action lowers pH NQO1 is maintained in *Enz-FADH*$_2$ shape with the Redox level by L-glucose L-glucose maintains the shape but blocks the NAD(P)H binding site of NQO1 Electron transfer along the microtubules is impeded L-Glucose increases tonicity, but with no ATP for H^+ NHE exporters
UHF (x20 min) x3 alternate Days➔ effects unsatisfactory	UHF resonance/heat are undetectable
. . . . .	. . . . .
2) Fasting (?), then insulin-induced hypoglycaemia	Glycolysis is minimized, so NAD(P)H &ATP production are minimal $NAD(P)^+$:NAD(P)H falls below Redox threshold for NQO1 (*Enz-FADH*$_2$)
Infusion of L-glucose i.v. & D-Glucose infusion i.v.	Glycolysis is re-activated Production of ATP & NAD(P)H &NADH resumed by glycolysis
UHF (x20 min) x3consecutive days➔ effects desirable	Oxidative environment (*Enz-FAD*)Ratio $NAD(P)^+$:NAD(P)H rises above NQO1 Redox threshold (*Enz-FAD*)
UHF resonance &heat are detectable	NAD(P)H &NADH bind NQO1 & transfer electrons along microtubules
	L-Glucose provides hypertonic cytoplasm + ATP ➔NHE activated pH rises (desirable)
	Final Redox, NAD(P)H & NADH supplies are greater than at the start
Treatment is more gratifying	More fuel for the UHF resonance
	UHF resonance and heat production are more pronounced

THE UHF RESONANCE

NADPH: The basic hypothesis attempting to explain the UHF resonance effects proposed that electrons were drawn principally from NADH and NADPH, with the production of NAD^+ and $NADP^+$. Some of the effects of the NAD^+ in the cytoplasm have been mentioned, notably the stimulation of PARP groups and SIRTs (Traill, 2023). The latter may suppress the formation of cortical actin and the attachment s of cadherin molecules.

NAD^+-dependent SIRT2 & SIRT5 deacetylate and activate G6PD, potentially increasing cytoplasmic NADPH and hence antioxidant capacity (Wang *et al.,* 2014). With UHF applied, the new NADPH may feed more electrons to the resonance process.

Cancer Killing. How this occurs is unclear still – the UHF-generated oxidizing environment may damage important structures such as enzymes, structural proteins, microtubules and the motors. There are research opportunities.

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