An investigation into the effects of high dilution quinones on peripheral blood leukaemic lymphocyte metabolism.

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Summary

This *in vitro* investigation attempts to see if cultured leukaemic peripheral blood lymphocytes from children with acute lymphoblastic leukaemia ("ALL") can be corrected by introducing high redox potential quinones into the culture medium.

Modern treatments for ALL aim to kill the aberrant cells with minimal effect on normal cells, and perhaps some 70 percent of such patients are restored to health (Stiller, 2004). Inevitably however there are side effects such as hair loss, pain, and subsequently lower IQ levels. In a recent Hungarian clinical trial on colorectal cancer patients (see below) with certain quinone structures such side effects were minimal and metastases were dramatically reduced. Should results of this *in vitro* study also be found positive, and clinical trials likewise the treatment may ultimately prove adjuvant in reducing the adverse side effects of present interventions.

Introduction

Albert Szent-Gyorgyi, like Otto Warburg some decades earlier (Warburg, 1930, 1956), proposed that cancer could be regarded as a disorder of cellular metabolism and that its correction could be achieved using high redox potential quinones (which have active para and ortho carbonyl groups) or serial carbonyls such as glyoxal (Szent-Gyorgyi, 1979,1982)

This idea lay dormant until the early 1990s when a Hungarian group, following his suggestions, produced a biomolecule by fermentation of wheat germ, *parabenzoquinone*, which in high dilution proved highly effective in stimulating the immune responsiveness in mice (Hidvegi, Raso et al.,1999), in ameliorating the clinical manifestations of experimental systemic lupus erythematosis in naïve mice, (Ehrenfeld, Blank et al., 2001), in inducing apoptosis in MCF7 breast cancer cells (Tompa, Kocsis et al., 2001) then in a trial of experimental colon carcinogenesis in F344 rats (Zalatnai, Lapis et al., 2002) and later in a clinical trial of colorectal cancer patients (Jakab, Shoenfeld, et al., 2003) . Equal success has recently been achieved with lung cancer and breast cancer patients, but no study has yet examined its potential in the treatment of leukaemia.

A common feature of cancer cells is an avidity for glucose and disinterest in molecular oxygen. There is also a dearth of glycoproteins on their plasma membrane surfaces, with loss of regulatory growth control as a consequence. There is some evidence that leukaemic lymphocytes also have reduced glycoprotein expression (Wuchter, Ruppert et al., 2000; Smolenska-Sym, Zdebska et al., 1998). This study series investigates the effects of parabenzoquinone in high dilution

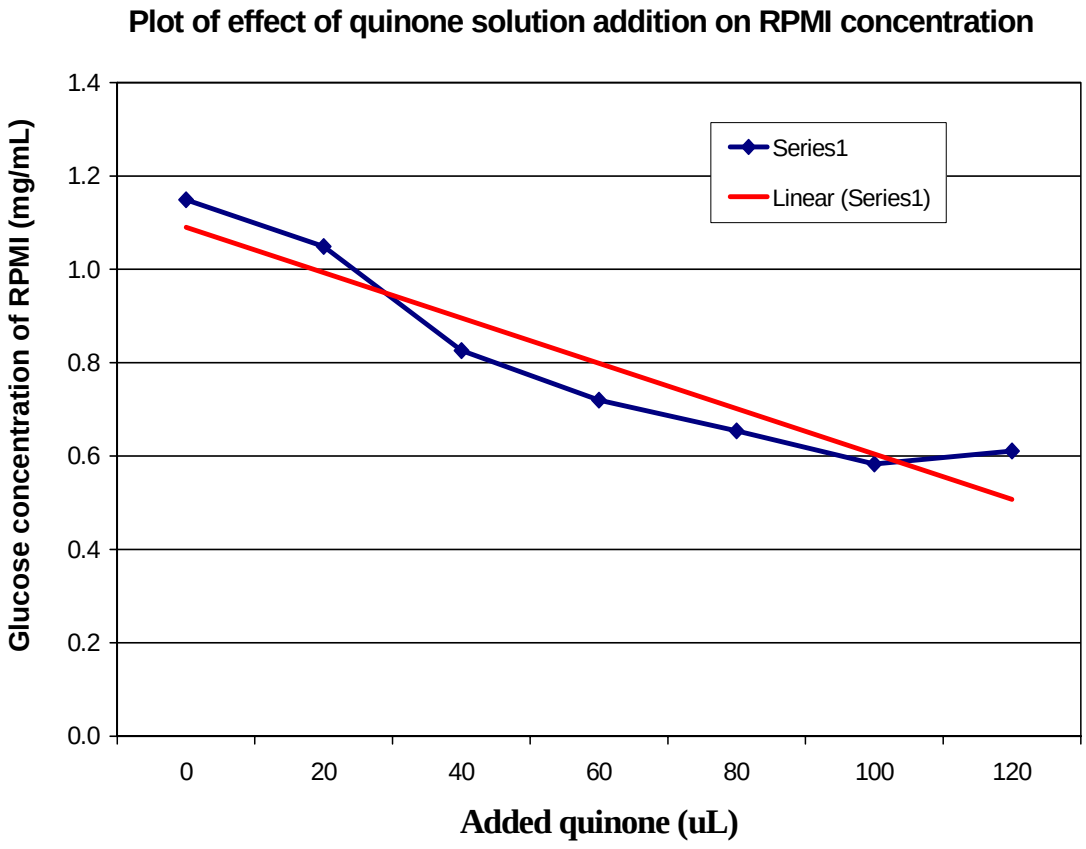
a) on glucose uptake and
b) on respiration,

on the premise that leukaemic lymphocytes exhibit altered metabolism, and that both these are markers for a return to normal metabolism and reversion to the oxidative phosphorylation pathway.

Materials and Methods

a) The Glucose Assays

Peripheral blood lymphocytes ("PBLs") drawn from children diagnosed with acute lymphoblastic leukaemia are isolated by centrifugation, washed twice in Dulbecco's and cultured in nutrient medium (RPMI 1640 without phenol red) and without antibiotics or mycotics. The culture medium is standardised and separated into two samples of 100microlitres. To one of these is added 10, 20, or 50 microlitres of parabenzoquinone diluted -10^6 times. After 3, 12 and 24 hours incubation at 37° C the samples are assayed for glucose level by a) glucose oxidase ("GO") and hexokinase ("HK") assays using spectrophotometry at 340nm (Sigma Diagnostics, Poole Dorset). The results are assessed statistically to see whether the addition of the quinone has lowered the level of glucose uptake by the cells. The assays are conducted blind and repeated three times for each culture.



b) *The Respiration assay*

To investigate whether the addition of parabenzoquinone in high dilution to the cultured PBLs has the effect of increasing the molecular oxygen uptake by the cells, a Clarke oxygen electrode is used. Cells are plated onto a c 1.5cm circular substrate placed in the water-jacketed chamber of the electrode and maintained at 37° C by means of a waterbath monitored by thermistors and datalogged, and the progress of the oxygen level monitored

polarigraphically both in the quinone-dosed and the non-dosed medium. As before, various levels of the diluted quinone are added to the solution and the assay is conducted three times for each culture.

Results

This work is ongoing at present using PBLs from cancer patients other than those diagnosed with leukaemia in order to prove and gain experience of the sensitivity of the assay systems and appropriate dose levels. The Chart below contains an example of the calculated vs. observed effect of added quinone on RPMI 1640 nutrient concentration.

Conclusion

A number of laboratory studies on cells and several clinical trials have indicated that parabenzoquinone is the active ingredient in successful interventions for a number of cancer subtypes. This *in vitro* study aims to see if the same benefits might obtain in cases of childhood leukaemia.

Discussion

The mechanism of interaction between quinone family molecules and cellular metabolism is best illustrated by the well-characterised biomolecule ubiquinone, which transports electrons and hydrogen protons separately to cytochrome b-c1 complex in the inner mitiochondrial membrane during oxidative phosphorylation. This quinone is encumbered with three methyl groups and a 10 five-carbon unit isoprenoid tail so that its redox potential is much lower than parabenzoquinone which lacks these substituents. Ubiquinone engages in an intermediate process during the electron delivery which turns it into a temporary free radical, ubisemiquinone, when it picks up a second electron and a concomitant hydrogen proton, which is shed when the electron is delivered (see Alberts, Bray et al., 1993). Delivery of the protons to one side and the electrons to the other side of the membrane creates a potential difference across the mitochondrial membrane in preparation for the binding of a third, energy-rich phosphate to adenosine diphosphate, an indispensable part of oxidative metabolism.

If a potential carcinogen such a toxic amine is blocking the ox-phos pathway by having bound to a pathway component, the free radical action of ubiquinone is normally sufficient to cleave it. On occasion however a superior dehydrogenator is required and the higher redox potential of parabenzoquinone, which is unencumbered by methyl groups or an isoprenoid tail, is better equipped for this dehydrogenating role.

A Hungarian group with laboratory experience of the reagent reports that it also downregulates the MHC1 proteins in tumour T and B cell lines (Fajka-Boka, Hidvegi et al., 2002). This effect is different from that proposed by Szent-Gyorgyi, since intuitively one would not expect apoptotic signals if the tumour cell has recovered its normal metabolism. Although the downregulation of MHC1 prevents killing by cytotoxic T cells, the tumour cells become susceptible to natural killer (NK) activity.

The Hungarian group advanced no hypothesis of how the reagent might act systemically. Early work however proposed that, in the presence of liberal molecular oxygen, peroxide free radical formation is achieved and acts in a chain reaction-like manner to dehydrogenate all other pathogens so as to permit the re-establishment of normal metabolism, hence the antimetastatic effects noted.

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