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# ANTIPROLIFERATIVE EFFECT OF SPIRONOLACTONE AGAINST 60 HUMAN CELL LINES RESULTS FROM THE NATIONAL CANCER INSTITUT'S ANTICANCER DRUG SCREEN

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**ABSTRACT**
In previous study we found the best cytotoxicity was on breast cancer cell line in mice (AMN-3) and human epidermoid larynx carcinoma (Hep-2) at high concentration 100,500,250µ/ml of spironolactone (SPL) .The NCI's results on human 60 cell lines, against SPL showed that there was CCRI-CEM cell line of leukemia is more sensitive towards the SPL than other cell lines of leukemia and all 60 human cell lines. All cell lines of colon cancer didn't show growth inhibition against SPL. The sensitivity of other cell lines were different against SPL. However SPL might be useful in the treatment of a variety of diseases depending on angiogenesis.

**KEYWORDS:** Spironolactone, human cell line, national cancer institute.

## INTRODUCTION

Spironolactone was first known to possess anti-inflammatory properties as early as 1961[1]. However that observation seemed to have gone largely unnoticed until the last few years. In a recent study, Japanese researchers looking for a reduction in cardiovascular risk factors related to inflammation found spironolactone to be the most potent anti-inflammatory medication they studied[2]. Specifically, it was found to potently reduce both TNF-alpha and MCP-1 in cultured human monocytes. These effects occurred at levels obtainable during routine oral administration of the medication, in a Danish population of rheumatoid arthritis patients (including Juvenile idiopathic arthritis), a modest dose of 1-3/kg /day resulted in a significant reduction of proinflammatory cytokines as well as decreased gene transcription for many regulators of inflammation[3].The aim of this article is to study the cytotoxic activity of spironolactone to 60 different human cell lines which is achieved with help of National Cancer Institute's anticancer screening program.

## MATERIALS &METHODS

Chemicals: the drug spironolactone which we had been used previously [4] submitted to National Cancer Institute (NCI) for testing the drug in a dose of ($10^{-5}$) against 60 human cell panels. The NCI's anticancer drug screen: the protocol used for cytotoxicity assessment in the NCI anticancer screening program has been described in detail [5]. Briefly, tumour cell lines derived from from leukemia, lung, colon, brain, melanoma, ovary, kidney, prostate, and breast were grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamin Cells (5000-40000 cells / well) were plated into 96-well micrititer plates and allowed to grow for 24 h at 37°C in a humnidified atmosphere supplemented with 5% $CO_2$ spironolactone , dissolved in dimethyl sulfoxide, was then added to the cells at final concentration 0.01µM , after which the cells were incubated for another 48 h. At the end of the incubation period, the cells were fixed in situ and stained with the protein –staining dye sulforhodamine B. After solubiliation of the dye, the optical density of the stain was measured at 515 nm. One dose-response parameters were calculated for the drug growth inhibition and lethality.

## RESULTS

The in vitro cytotoxicity parameters (growth percent and lethality) were obtained for spironolactone in NCI's anticancer screening program are listed in Figure 1 .The one dose data is reported as a mean graph of the percent growth of treated cells. The number reported for this assay is growth relative to the no-drug control, and relative to the time 0 number of cells. This allows detection of both growth inhibition (values between 0 and 100) and lethality (values less than 0). The percentage of growth inhibition is listed in Table1. Treatment with spironolactone yielded good response in percent of growth inhibition for CCRF-CEM cell line (18.1) in leukemia, other cell lines gave percent of growth inhibition ranged from 1 to 4.2in the same panel. In non-small cell lung cancer the greater response was with EKVX and NCI-H522 cell lines (10 and 7.4) respectively. The response of other cell lines in the same panel was weak; range was (0.4-2.6). All cell lines of colon cancer were not sensitive to SPL. Three cell lines in CNS cancer, SF295, SF-539 and SNB-19 showed percent of growth inhibition 6.7, 9.9 and 7 respectively. UACC-62 cell line in melanoma had greater response (16), other cell lines in melanoma; the range of percent of inhibition was (0.1-6.4). Ovarian ell lines showed range of percent of inhibition (2.2-4.6). The range of percent of growth inhibition in cell lines of renal cancer (1-13.6). Only one cell line PC-3 in prostate yielded percent of growth inhibition (5.4). In breast cancer the cell lines MCF7, BT-549 and T-470, the percent of growth inhibition was 5.4, 3.9 and 9 respectively.

**Developmental Therapeutics Program**

**One Dose Mean Graph**

| NSC: 150399 / 4 | Conc: 1.00E-5 Molar | Test Date: Feb 02, 2009 |
|---|---|---|
| Experiment ID: 0902OS52 | | Report Date: Sep 28, 2013 |

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 81.88 |
| HL-60(TB) | 98.99 |
| K-562 | 102.83 |
| MOLT-4 | 102.30 |
| RPMI-8226 | 95.81 |
| SR | 97.57 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 98.45 |
| EKVX | 90.03 |
| HOP-62 | 110.01 |
| HOP-92 | 98.10 |
| NCI-H226 | 98.44 |
| NCI-H23 | 106.84 |
| NCI-H322M | 97.36 |
| NCI-H460 | 99.56 |
| NCI-H522 | 92.56 |
| Colon Cancer | |
| COLO 205 | 102.40 |
| HCC-2998 | 107.11 |
| HCT-116 | 104.42 |
| HCT-15 | 111.99 |
| HT29 | 100.37 |
| KM12 | 104.40 |
| SW-620 | 103.65 |
| CNS Cancer | |
| SF-268 | 104.38 |
| SF-295 | 93.32 |
| SF-539 | 90.15 |
| SNB-19 | 93.04 |
| SNB-75 | 105.22 |
| U251 | 105.25 |
| Melanoma | |
| LOX IMVI | 99.94 |
| MALME-3M | 114.68 |
| M14 | 113.30 |
| MDA-MB-435 | 93.64 |
| SK-MEL-2 | 110.66 |
| SK-MEL-28 | 108.68 |
| SK-MEL-5 | 96.19 |
| UACC-257 | 95.75 |
| UACC-62 | 83.97 |
| Ovarian Cancer | |
| IGROV1 | 95.36 |
| OVCAR-3 | 97.78 |
| OVCAR-4 | 96.32 |
| OVCAR-5 | 113.36 |
| OVCAR-8 | 105.64 |
| NCI/ADR-RES | 113.20 |
| SK-OV-3 | 97.72 |
| Renal Cancer | |
| 786-0 | 110.08 |
| A498 | 113.38 |
| ACHN | 97.92 |
| CAKI-1 | 99.00 |
| RXF 393 | 116.66 |
| SN12C | 98.28 |
| TK-10 | 99.61 |
| UO-31 | 86.38 |
| Prostate Cancer | |
| PC-3 | 96.04 |
| DU-145 | 111.35 |
| Breast Cancer | |
| MCF7 | 94.60 |
| MDA-MB-231/ATCC | 107.35 |
| HS 578T | 121.92 |
| BT-549 | 96.06 |
| T-47D | 91.03 |
| MDA-MB-468 | 106.92 |
| Mean | 101.32 |
| Delta | 19.44 |
| Range | 40.04 |

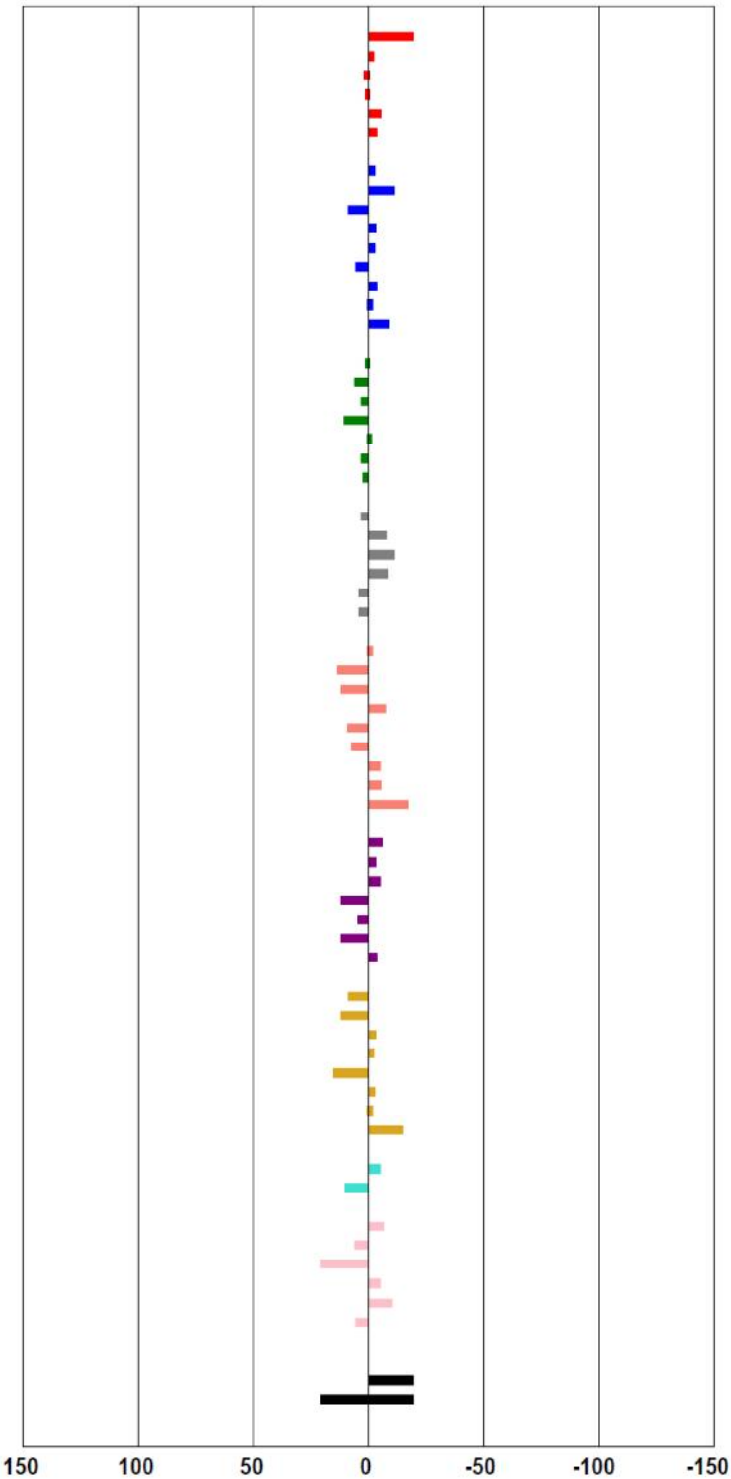


**FIGURE 1.** Growth percent and lethality of 60 human cell lines produced by one dose ($10^{-5}$M) of spironolactone (NCI's results).

**TABLE 1:** Percent of growth inhibition of cell lies in different type tissue

| Cell type | Tissue type | % of growth inhibition |
|---|---|---|
| CCRF-CEM | Leukemia | 18.1 |
| HL-60(TB) | Leukemia | 1 |
| PRMI-8226 | Leukemia | 4.2 |
| SR | Leukemia | 1.8 |
| A549/ATCC | Non-small cell lung cancer | 1.6 |
| EKVX | Non-small cell lung cancer | 10 |
| HOP-92 | Non-small cell lung cancer | 1.9 |
| NCI-H226 | Non-small cell lung cancer | 1.9 |
| NCH-H226 | Non-small cell lung cancer | 1.6 |
| NCI-H322M | Non-small cell lung cancer | 2.6 |
| NCI-H460 | Non-small cell lung cancer | 0.4 |
| NCI-H522 | Non-small cell lung cancer | 7.4 |
| SF-295 | CNS cancer | 6.7 |
| SF-539 | CNS cancer | 9.9 |
| SNB-19 | CNS cancer | 7 |
| OXIMV | Melanoma | 0.1 |
| MDA-MB-435 | Melanoma | 6.4 |
| SK-MEL-5 | Melanoma | 3.8 |
| UACC-257 | Melanoma | 4.3 |
| UACC-62 | Melanoma | 16 |
| IGROV1 | Ovarian cancer | 4.6 |
| OVCAR-3 | Ovarian cancer | 2.2 |
| OVCAR-4 | Ovarian cancer | 3.7 |
| SK-OV-3 | Ovarian cancer | 2.3 |
| ACHN | Renal cancer | 2.1 |
| CAKI-1 | Renal cancer | 1 |
| SNI2C | Renal cancer | 1.7 |
| Tk-10 | Renal cancer | 0.4 |
| UO-31 | Renal cancer | 13.6 |
| PC-3 | Prostate cancer | 5.4 |
| MCF7 | Breast cancer | 5.4 |
| BT-549 | Breast cancer | 3.9 |
| T-470 | Breast cancer | 9 |

**DISCUSSION**

In previous study we found a clear cytotoxic activity of pure spironolactone with high significance in a two cancer cell lines during the three exposure time, suggesting that the cytotoxic effect of pure spironolactone is a dose and time dependent. The best cytotoxic activity was on AMN-3 and Hep-2 cell lines at the high concentration 1000, 500, 250 µg/ml. The results also suggest that ANN3 cell line is the most sensitive cancer –cell line to pure spironolactone than Hep-2 cell line after 24-hr. of exposure to drug [4].

The NCI results showed that most of cell lines of leukemia panel showed growth inhibition against SPL (Fig.1 and Table-1) CCRI-CEM cell line of leukemia is more sensitive towards the spironolactone than other cell lines of leukemia. The HL-600 (TB) cell of the same panel was least sensitive to the drug. However the percentage of growth inhibition of CCRI-CEM cell line was the greatest among all 60 human cell lines. EKVX cell line of non-small lung carcinoma showed the highest sensitivity against SPL in the same panel while CH-H226 cell type the growth inhibition was the least. Spiranolactone inhibits angiogenesis directly through blockade of vascular endothelial cells from responding to a wide spectrum of angiogenesis stimulations, including Vascular Health Growth Factor (VEGF) and Basic Fibroblast Growth Factor (bFGF). As a result, the abilities of endothelial cell on proliferation cells are suppressed, and angiogenesis is inhibited[6]. SF-539 cell line of ovarian cancer is the most responsive than other cell lines of the same tissue, while the OVCAR-3 cell line gave a least percentage of growth inhibition. The sensitivity of ovarian cancer cell line panels against SPL is of importance because there is a need for new chemotherapeutic drugs effective against ovarian cancer [7]. Uo-31 cell line of renal cancer showed sensitivity against SPL than other cell line of renal cancer is the least sensitivity. Spiranolactone is an aldosterone antagonist, aldosterone present in culture media used for cell proliferation, and this study when aldosterone added to the media showed no effect on Bovine Capillary Endothelial Cell (BCEC) proliferation.It is therefore unlikely that SPL is inhibiting angiogenesis through its antimilanocorticoid effect, thus implicating a mechanism different from that of angiostatic steroids [8]. PC-3 cell line is the only type which showed sensitivity in the prostate cancer cell line panel.T-470 cell line of breast cancer is most sensitive towards SPL and BT-459 showed the least sensitivity. Estrogen stimulates breast cancer cell line proliferation and their effects are mediated by the estrogen receptor (ER). In contrast SPL suppressed the effects of androstenedion-induced cell growth by antiandrogens

effect [9] through inhibition of 5-alpha-dihydrotestosterone binding to cytosolic androgen receptor[10] and exert their anti-proliferative effect by interaction with androgen receptor (AR)[11]. This drug might be useful in the treatment of a variety of diseases dependent on angiogenesis, such as solid tumour growth and macular degeneration. It can be synthesized much further compounds derived from SPL itself as prodrug to find out a compound more potent and safer.

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