VANDERBILT UNIVERSITY MEDICAL CENTER

- Human ovarian cancer cell culture study-

Evaluation of the Effects of Carnitine, Doxorubicin and Combinations Thereof on Human Ovarian Carcinoma

The purpose of the study was to evaluate doxorubicin (trade name ADRIAMYCIN), carnitine (L-carnitine) and combinations thereof at incremental dosages. The incremental dosages were tested using human ovarian carcinoma or cancer cells, SKOV3 cells, in culture. This approach more closely mimics the human condition. In addition, the effect of each dosage regimen was evaluated to determine a) effect of each active agent on cell viability by its kill capacity and b) whether there are advantages to the combination of active agents.

Cells and Method:

SKOV3 cells ($2X10^3$ cells/well) were seeded in 96-well plates and grown in McCoy's 5a media containing 10% fetal bovine serum, 100 units/mL of penicillin, and 100 µg/mL streptomycin for 15 hours. Media was replaced to fresh media with various doses of doxorubicin and/or L-carnitine base and cells were kept for another 72 hours. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) colorimetric assays per manufacturer's instructions (Promega of Madison, WI) were monitored at an absorbance of 490 nm as an index of cell viability. Experiments were run in triplicate. Data are shown as mean ± SD.

Results:

In Figure 1, various doses and effects on tumor cell viability for doxorubicin (ADRIAMYCIN™), L-carnitine, and the combination of doxorubicin and L-carnitine are shown. In Figure 2, various doses and effects on tumor cell viability of doxurubicin alone are shown. In each of the three treatment groups, there was a consistent dose response.

In this statistical analysis, the drug regimens that would kill at least 50 percent of the tumor cells were evaluated, and are shown in Figure 3. The results of the drug regimens are shown below in Table 1.

Table 1

Treatment Drug (Dose)	% Viability Mean {95% CI}	P-value Code
Doxorubicin (10 nM)	4.5 {-5.2, 14.2}	A
Doxorubicin (1 nM) and L-Carnitine (250 uM)	29.8 {22.3, 37.3}	B
L-Carnitine (250 uM)	40.1 {29.8, 50.5}	C
Doxorubicin (0.1 nM) and L-Carnitine (250 uM)	45.7 {39.0, 52.4}	D
Doxorubicin (1 nM)	46.5 {44.6, 48.4}	E
L-Carnitine (10 uM)	75.6 {55.5, 95.8}	F
L-Carnitine (0 uM)	78.3 {46.2, 110.5)	G
Doxorubicin (0 nM)	104.3 {77.3, 131.3}	H

As expected, the % viability mean of doxorubicin (10 nM) was significantly higher than baseline doxorubicin (0 nM) (A vs. H, $p<0.0001$) as shown in Table 1. The % viability mean of L-Carnitine (250 μM) also was significantly higher than baseline doxorubicin (0 nM) (C vs. H, $p<0.0001$) as shown in Table 1.

The data for drug regimens exhibiting killing >50% of tumor cells are shown in Table 2 below.

Table 2

Treatment Drug (Dose)	% Viability Mean {95% CI}	P-value Code
Doxorubicin (10 nM)	4.5 {-5.2, 14.2}	A
Doxorubicin (1 nM) and L-Carnitine (250 uM)	29.8 {22.3, 37.3}	B
L-Carnitine (250 uM)	40.1 {29.8, 50.5}	C
Doxorubicin (0.1 nM) and L-Carnitine (250 uM)	45.7 {39.0, 52.4}	D
Doxorubicin (1 nM)	46.5 {44.6, 48.4}	E

The % viability mean of the combination of doxorubicin (1 nM) and L-carnitine (250 μM) was significantly better than L-carnitine (250 μM) (B vs. C, p=0.001) and doxorubicin (1 nM) (B vs. E, p=0.001) as shown in Table 2. Both doses (B & D) of doxorubicin and L-carnitine reduced cell viability below 50%.

For each of the drug regimens exhibiting killing >50% of tumor cells (see Table 2), doxorubicin (10 nM) was significantly better than the four other regimens (A vs. B, C, D, E, p=0.0001). However, doxorubicin (1 nM) and L-carnitine (250 μM) also was significantly better at killing tumor cells than the other three regimens (B vs. C, D, E, p=0.001).

The results of this study indicate that: 1) carnitine alone demonstrated statistically significant tumor kill capacity by reducing tumor cell viability, b) the

combination of carnitine and doxorubicin has greater tumor kill capacity than either alone, and c) carnitine increases doxorubicin's tumor kill capacity both at low and higher doses of doxorubicin.

The foregoing discussion discloses and describes merely exemplary embodiments of the present invention. One skilled in the art will readily recognize from such discussion, and from the accompanying drawings and claims, that various changes, modifications and variations can be made therein without departing from the spirit and scope of the invention as defined in the following claims.

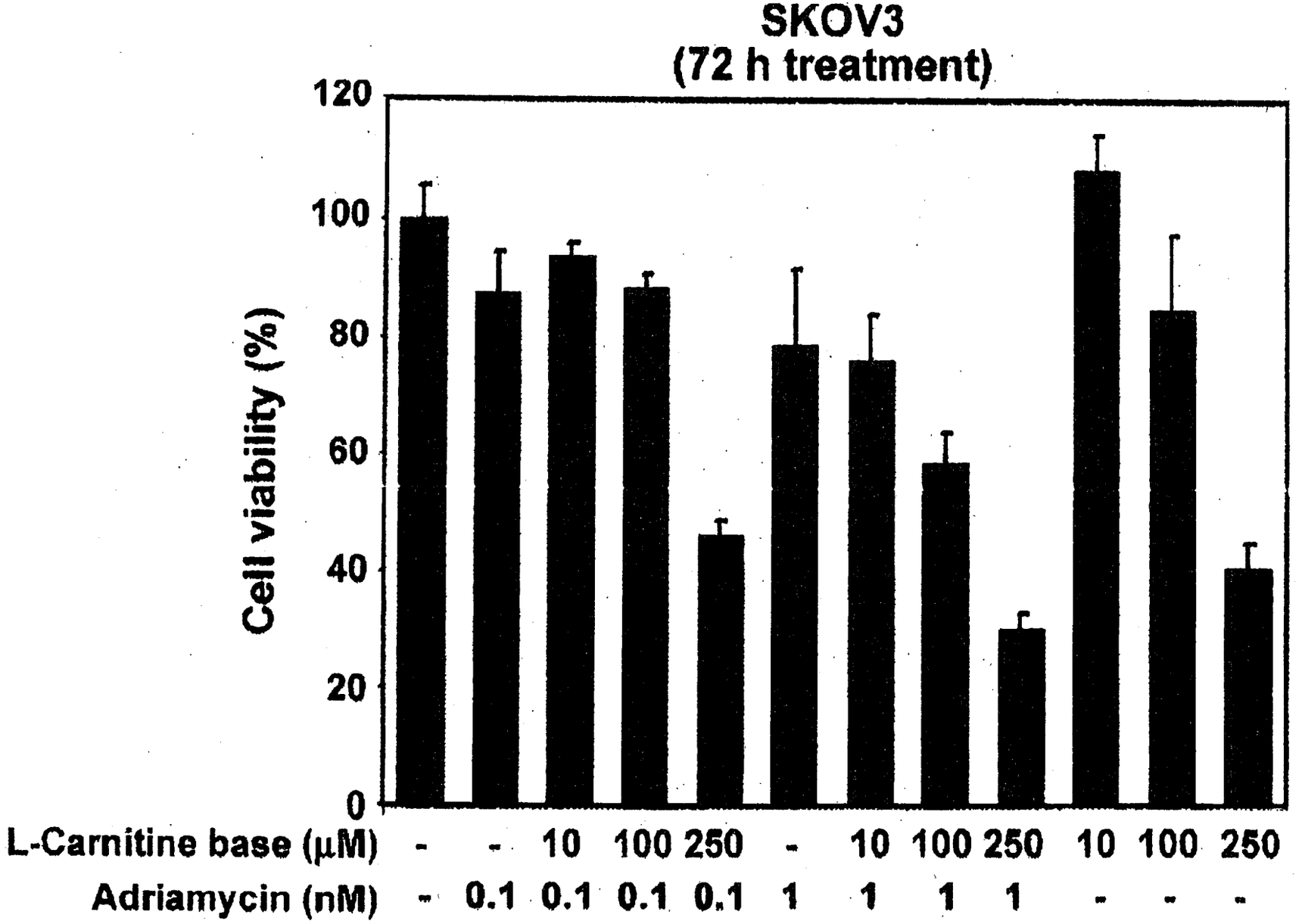

SKOV3
(72 h treatment)
Cell viability (%)
120
100
80
60
40
20
0
L-Carnitine base (µM) - - 10 100 250 - 10 100 250 10 100 250
Adriamycin (nM) - 0.1 0.1 0.1 0.1 1 1 1 1 - - -

FIG. 1

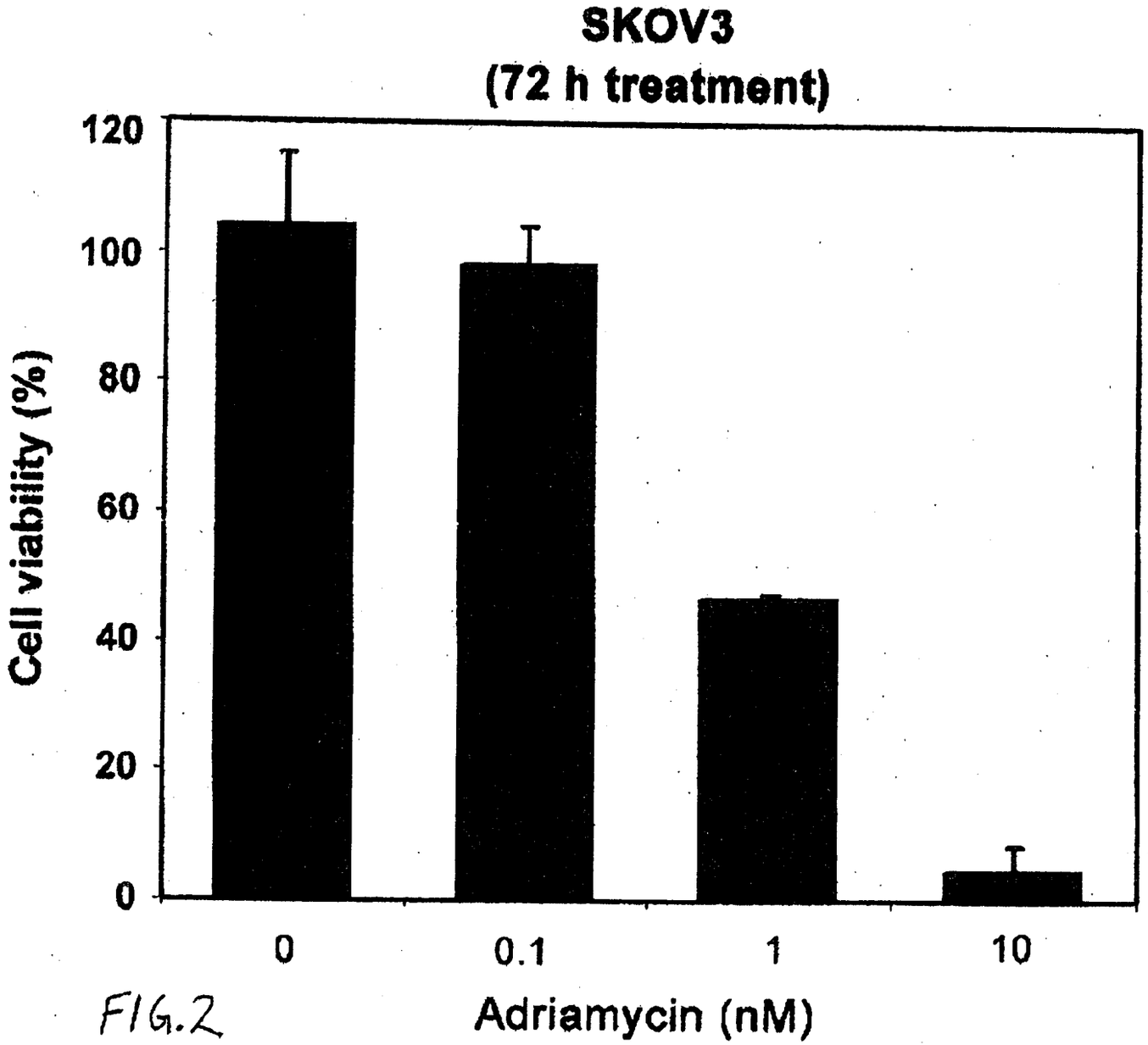
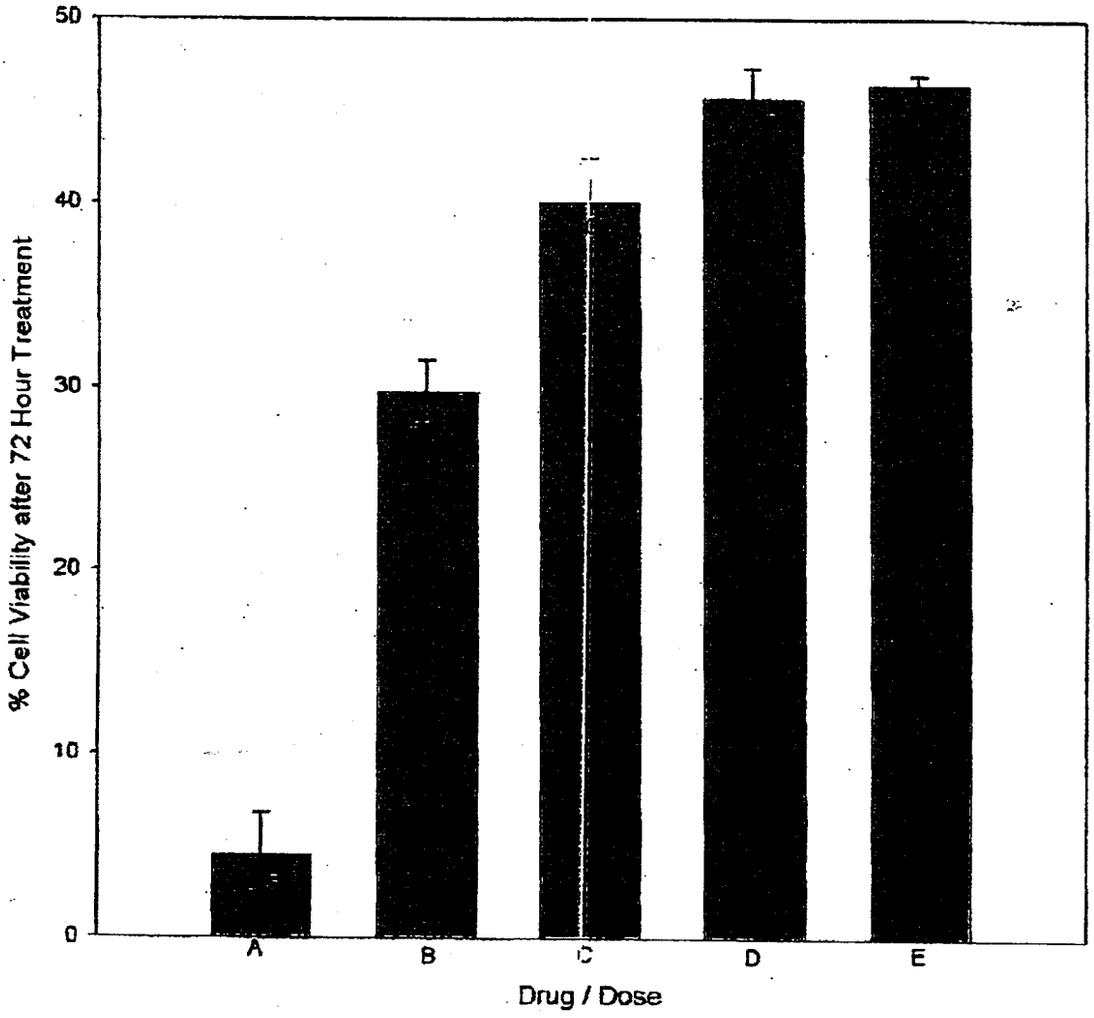

SKOV3
(72 h treatment)
Cell viability (%)
120
100
80
60
40
20
0
0
0.1
1
10
Adriamycin (nM)

FIG.2

Figure 3: *In Vitro* Reduction of Ovarian Cancer Cell Viability for Adriamycin Alone, L-Carnitine Alone, and Drug/Dose Combinations



A=Adriamycin (10nM) B=Adriamycin (1 nM) + L-carnitine (250uM) C=L-carnitine (250uM)
D=Adriamycin (0.1nM) + L-carnitine (250uM) E=Adriamycin (1nM)

FIG. 3