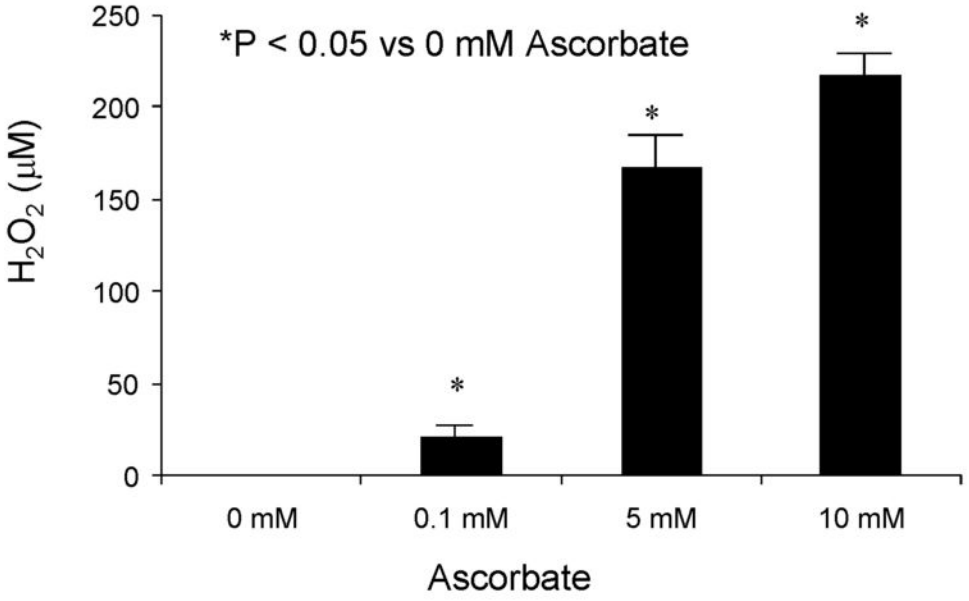
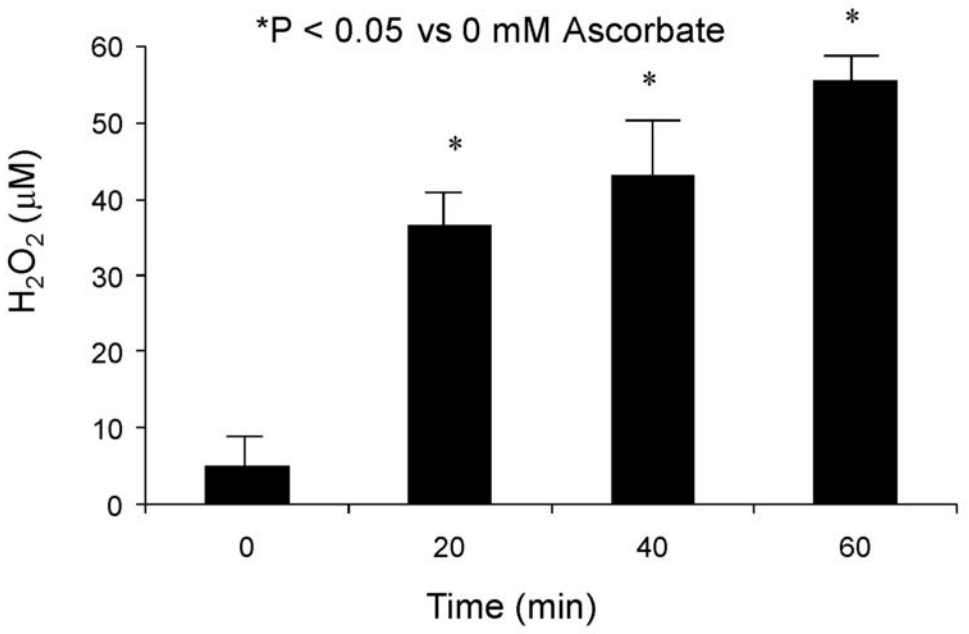
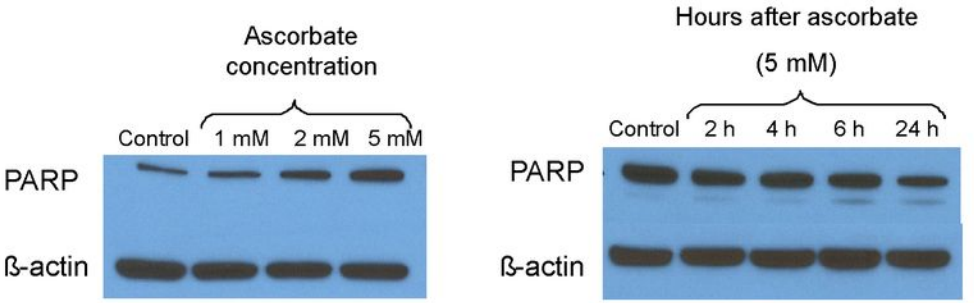
A.


*P < 0.05 vs 0 mM Ascorbate
H2O2 (μM)
250
200
150
100
50
0
*
*
*
*
0 mM
0.1 mM
5 mM
10 mM
Ascorbate

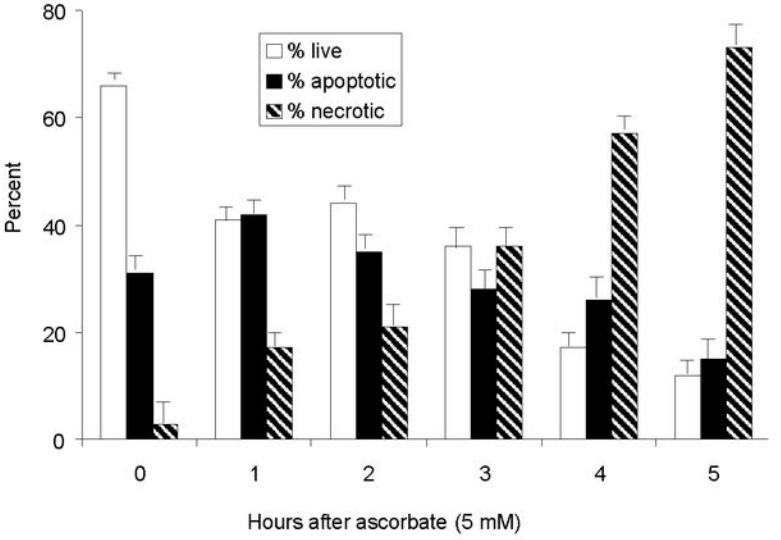
B.


*P < 0.05 vs 0 mM Ascorbate
H2O2 (μM)
60
50
40
30
20
10
0
*
*
*
0
20
40
60
Time (min)

A.


Ascorbate concentration
Control
1 mM
2 mM
5 mM
PARP
ß-actin
Hours after ascorbate (5 mM)
Control
2 h
4 h
6 h
24 h
PARP
ß-actin

B.


% live
% apoptotic
% necrotic
Percent
80
60
40
20
0
0
1
2
3
4
5
Hours after ascorbate (5 mM)

Supplemental figures

Supplemental Figure 1. Ascorbate increases H_2O_2 generation in cell culture medium. H_2O_2 was measured by oxygen electrode. A. H_2O_2 increased as a function of ascorbate concentration. Ascorbate (0.1, 5.0, and 10.0 mM) increased H_2O_2 (20, 167, and 217 μM, respectively). B. H_2O_2 increased as a function of time. Ascorbate (1 mM) increased H_2O_2 concentrations to over 50 μM after 60 min of incubation.

Supplemental Figure 2. Ascorbate-induced cytotoxicity is a non-caspase mediated cell death. A. Minimal change is seen in PARP cleavage in either a dose-dependent or time-dependent manner following treatment with ascorbate. MIA PaCa-2 cells were treated with ascorbate (1-5 mM for 1 h) or with ascorbate (5 mM for 1 h). Cells were harvested up to 24 h and equal amounts of protein were separated on 15% SDS-PAGE and immunoblotted. The 116 kDa band represents intact PARP and the 85 kDa band represents the cleaved PARP. Data represent results from three separate experiments. B. Necrotic cells increased over time following ascorbate (5 mM). Annexin/propidium iodide staining measured by flow cytometry demonstrates an initial increase in the apoptotic fraction of cells one hour after ascorbate treatment. However, the necrotic fraction of cells increased in a time-dependent manner following treatment with ascorbate.