CCRC 60104) and human hepatoblastoma cells (HepG2, CCRC 60025) were obtained from the Culture Collection and Research Center in Taiwan. Both lines of cells were grown as a monolayer at 37 °C in 95% air and 5% CO_2 in DMEM supplemented with 10% fetal bovine serum and routinely subcultured twice a week. I3C and ICZ were dissolved in DMSO, and the DMSO concentration added to the media never exceeded 0.1% (v/v). BNF served as a positive control.

Cytotoxicity Assays

The cytotoxic effects of ICZ were examined by a CellTiter 96 Aqueous One Solution Cell Proliferation Assay@ Kit (Promega, Madison, WI). Basically, 10^4 cells were treated with various concentrations of ICZ, and cells were harvested 24 h later. Live cells convert 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) to a formazan dye that can be detected at $OD_{492\ nm}$ by a microplate reader.

Assays for GST Enzyme Activity

After incubation with the chemicals, cells were collected by scraping and lysed by potassium phosphate buffer containing 0.5% Triton-100. The cytosolic fractions resulting from ultracentrifugation (100,000 xg, 60 min at 4 °C) were used for the GST enzyme assay. Cytosolic GST enzyme activity was determined spectrophotometrically at 25 °C using CDNB as the substrate according to the method described by Habig et al.[17] In brief, the assay was carried out by mixing the cytosol with the reaction mixture which contained 100 mM potassium phosphate buffer (pH 6.5), 1 mM CDNB, and 1 mM GSH, and the formation of the CDNB conjugate was determined at $OD_{340\ nm}$. Protein concentration of the cytosol was determined by the Bradford method. using the Bio-rad dye system, with bovine serum albumin serving as a standard. The GST enzyme activity was expressed as nmol CDNB conjugate formed/min/mg protein.

SDS-PAGE and Western Blotting

To determine whether the increased GST enzyme activity was due to increased expression of GST isoenzyme proteins, Western blot analysis was performed. Twenty micrograms of cytosolic protein was separated by 15% sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (SDS-PAGE) using an SDS-polyacrylamide gel system (GIBCO). The proteins were then electroblotted onto a nitrocellulose membrane using a semidry blotting system, and the blots were treated with antibodies against human GST-α, GST-π, and GST-μ, respectively. After the blot was incubated with peroxidase-conjugated IgG (Jackson Immuno Research Laboratories, PA), specific bindings of these antibodies were detected using the enhanced chemiluminescent ECL Western detection system (Amersham Life Science).

Statistical Analysis

Values are expressed as the means ± SD. One-way ANOVA followed by Duncan's test was used to determine the statistical differences between groups using the SAS software vers. 6.12 (SAS Institute, Cary, NC). Significance of mean differences was based on a p value of < 0.05.

RESULTS

Effects of ICZ on Total GST Activities in Hepatoma Cells

To examine the effects of ICZ on total cellular GST activities in hepatoma cells, time-course and concentration-dependent experiments were performed. In Hepa-1 cells, we observed that total GST activity was highest after 24 h of treatment with ICZ (data not shown), so 24 h was selected for the following experiments. Fig. 2 indicates that the GST activity could be enhanced by ICZ at a concentration as low as 3 nM in Hepa-1 cells after 24 h of treatment. The induction of GST activity increased 9% over that of the DMSO control after 3-nM ICZ treatment, and reached a maximum at a concentration of 3 μM (a 25% increase) in Hepa-1 cells after 24 h of treatment. The maximal induction potency was comparable to the BNF positive control, which caused a 23% increase over the DMSO control. On the contrary, no such enhancement was observed in HepG2 cells after ICZ