ophilic xenobiotics from the organism. GSTs are a family of dimeric isoenzymes that catalyze the conjugation of glutathione (GSH) with electrophilic intermediates generated from phase I reactions and thus prevent the carcinogenic process. The most abundant mammalian GST isoenzymes have been classified into 3 major groups, namely, α, μ, and π, with molecular weights of 24-29 kDa.[5]

Cruciferous vegetables including cabbage, Napa cabbage, cauliflower, broccoli, and Brussels sprouts have received great attention as potential chemoprotectors. Epidemiological studies have demonstrated that increased consumption of cruciferous vegetables is associated with a reduction in cancers of the colon and rectum.[5,6] Many animal studies have suggested that the indoles, such as indole-3-carbinol (I3C), in these vegetables are responsible for the chemopreventive effect and modification of the xenobiotic-metabolizing enzymes has been proposed as the mechanism.[4,8] I3C is one of the indolic compounds present in large quantities in cruciferous vegetables. Under acidic conditions, I3C can be further converted to various oligomeric products including indolo[3,2-*b*]carbazole (ICZ). Fig. 1 shows the structures of I3C and ICZ. It has been reported that ICZ can be detected in gastric and intestinal contents following oral administration of I3C.[9,10] In addition, ICZ could be found in feces of human subjects fed a normal diet and rats fed a purified diet.[10] Since we are constantly exposed to ICZ through our diet, it is important to characterize the physiological roles of ICZ in the body.

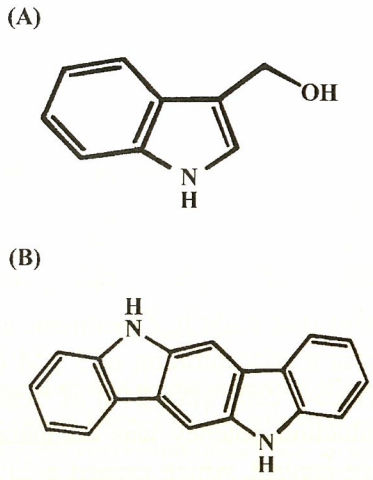

Fig. 1. Structures of indole-3-carbinol (A) and indolo[3,2-*b*]carbazole (B).

It has been reported that ICZ is a CYP1A1 inducer both in rats and in cultured cells.[10,11] Its induction activity is mainly due to its high binding affinity to the aryl hydrocarbon (Ah) receptor, a cytosolic protein associated with the expression of various xenobiotic-metabolizing enzymes,[12,13] including CYP1A1, quinone reductase, and GST. Cruciferous vegetables and I3C have been found to enhance phase II enzymes including the GST enzyme in animals and in cultured cells,[14-16] but it is not clear what compound is responsible for such induction. Because the murine hepatoma cell line (Hepa-1) is a good model to study the expression of xenobiotic-metabolizing enzymes, and because the induction of the GST gene is partially an AhR-dependent process, the effects of ICZ on the regulation of GST activity in Hepa-1 cells were studied. At the same time, the effect of ICZ on GST activity of a human hepatoma cell line (HepG2) was also studied.

MATERIALS AND METHODS

Chemicals and Biochemicals

ICZ was kindly provided by Dr. Bjeldanes (Univ. of California, Berkeley, CA). β-Naphthoflavone (BNF) was from Aldrich (Milwaukee, WI). Dulbecco's Modified Eagle medium (DMEM), fetal bovine serum, and the acrylamide gel system were purchased from GIBCO (Grand Island, NY). Glutathione (GSH), chloro-2,4-dinitrobenzene (CDNB), bovine serum albumin, and dimethyl sulfoxide (DMSO) were obtained from Sigma Chemical (St. Louis, MO). ECL Western blotting detection reagent was from Amersham Life Science (Cleveland, OH). Rabbit anti-human polyclonal GST-α, GST-μ, and GST-π antibodies were from Novo Castra (Newcastle, UK). All other laboratory chemicals were of the highest quality available and were purchased from Sigma Chemical, USB (Cleveland, OH), and Amersham Life Science.

Cell Culture

Murine hepatoma cells (Hepa-1c1c7, Hepa-1,