Available online at www.sciencedirect.com
ScienceDirect
Food and Chemical Toxicology 46 (2008) 761–766
www.elsevier.com/locate/foodchemtox

Effect of diisopropanolamine upon choline uptake and phospholipid synthesis in Chinese hamster ovary cells

W.T. Stott *, K.M. Kleinert

Toxicology and Environmental Research and Consulting, Building 1803, The Dow Chemical Company, Midland, MI 48674, United States

Received 18 January 2007; accepted 1 October 2007

Abstract

Aminoalcohols differ in mammalian toxicity at least in part based upon their ability to alter the metabolism of phospholipids and to cause depletion of the essential nutrient choline in animals. This study examined the incorporation of diisopropanolamine (DIPA) into phospholipids (PLs) and effects of DIPA upon choline uptake and phospholipid synthesis in Chinese hamster ovary (CHO) cells. Results were compared to those of a related secondary alcohol amine, diethanolamine (DEA), whose systemic toxicity is closely associated with its metabolic incorporation into PLs and depletion of choline pools. DIPA caused a dose-related inhibition of ^{3}H-choline uptake by CHO cells that was approximately 3–4 fold less potent, based upon an IC50, than that reported for DEA. DIPA, in contrast to DEA, did not cause changes in the synthesis rates of ^{33}P-phosphatidylethanolamine, ^{33}P-phosphatidylcholine or ^{33}P-sphingomyelin at either non-toxic or moderately toxic concentrations. Only approximately 0.004%, of administered ^{14}C-DIPA was metabolically incorporated into PLs, over 30-fold less than the incorporation of ^{14}C-DEA under similar conditions. Overall, these data and previous pharmacokinetic and toxicity data obtained *in vivo* suggests that DIPA is distinct from DEA and lacks significant choline and PL metabolism related toxicity in animals.

Keywords: Diisopropanolamine; DIPA; Choline; Phospholipid synthesis

1. Introduction

Diisopropanolamine (DIPA) is a member of a group of alcohol amines that are used in a variety of industrial and consumer product applications and which may vary widely in their toxicity to laboratory animals. The potential of several alcohol amines to alter the cellular uptake and metabolism of the essential nutrient choline and to alter phospholipid (PL) synthesis has been well recognized (Akesson, 1977; Barbee and Hartung, 1979; Glaser et al., 1974; Lerner, 1989). More recently, the toxicity of the secondary ethanol amine analogue of DIPA, diethanolamine (DEA) has come under scrutiny. DEA displays a multiple organ spectrum of toxicity in test rodents ranging from anemia to demyelination of central nervous system tissue to promotion of liver tumors (Melnick et al., 1994a,b; NTP, 1999). The toxicity of DEA has been directly related to its inhibition of choline uptake and subsequent deficiency, and to metabolic incorporation into headgroups of phospholipids (PLs) with subsequent alterations in membrane function (Barbee and Hartung, 1979; Lehman-McKeeman and Gamsky, 1999; Lehman-McKeeman et al., 2002; Leung et al., 2005; Mathews et al., 1995; Mendrala et al., 2001; Stott et al., 2000).

The repeated-dose toxicity of DIPA contrasts with that of DEA. As recently reviewed by Johnson et al., (in press), DIPA lacks significant systemic toxicity when administered via oral or dermal routes to rats for 13- and 4-weeks,

Abbreviations: CHO, Chinese hamster ovary; DEA, diethanolamine; DIPA, diisopropanolamine; NTP, National toxicology program; PBS, phosphate buffered saline; PL, phospholipid, ptdEA, phosphatidylethanolamine, PtdCho, phosphatidylcholine; SM, sphingomyelin; TLC, thin-layer-chromatography.

* Corresponding author. Tel.: +1 989 636 8203; fax: +1 989 638 9863.
E-mail address: wtstott@dow.com (W.T. Stott).

0278-6915/$ - see front matter © 2007 Published by Elsevier Ltd.
doi:10.1016/j.fct.2007.10.001

respectively. Only kidney weights are affected upon repeated imbibition of DIPA but this is not accompanied by histopathological changes at up to 1000 mg/kg/day. Also, no observable systemic toxicity has been reported in rats administered up to 750 mg/kg/day via dermal application, the highest dose level tested.

Despite the general lack of systemic toxicity of DIPA, however, it was not known whether it undergoes a similar metabolic fate as alcohol amines such as DEA or whether it can affect uptake of choline into cells. This study was undertaken to compare the potency of DIPA to that of DEA utilizing a cultured Chinese hamster ovary cell model *in vitro*.

2. Materials and methods

2.1. Test material

DIPA and DEA were obtained from The Dow Chemical Company, Plaquemine, Louisiana (purities greater than 99%). Diisopropanolamine-1-^{14}C hydrochloride was purchased from Sigma Radiochemicals (St. Louis, Missouri), choline chloride [methyl-^{3}H] and ^{33}P-orthophosphoric acid from Perkin–Elmer (Boston, Massachusetts), and ^{14}C-UL-diethanolamine from Wizard Laboratories (Davis, California). Radiochemical purities were greater than 99% and specific activities were 35 mCi/mmol for ^{14}C-DIPA, 86 Ci/mmol for ^{3}H-choline, 156.1 Ci/mg for ^{33}P-*ortho*-phosphonic acid, and 15 mCi/mmol for ^{14}C-DEA. All other biochemicals and cofactors were obtained from Sigma Chemical Company (St. Louis, Missouri).

2.2. Test organism and dosing

Chinese hamster ovary cells CHO-K_1-BH_4 were obtained from Dr. A. Hsie, Oak Ridge National Laboratory (Oak Ridge, Tennessee). CHO-K_1-BH_4 cells were cultured in 60 mm plastic plates or 20 mm wells in Ham's F-12 nutrient mix (LIFE TECHNOLOGIES, Grand Island, New York) supplemented with 5% (v/v) heat-inactivated (56 °C, 30 min), dialyzed fetal bovine serum (LIFE TECHNOLOGIES), antibiotics and antimycotics (penicillin G, 100 units/mL; streptomycin sulfate, 0.1 mg/mL; fungizone, 0.25 μg/mL; LIFE TECHNOLOGIES) and an additional 2 mM L-glutamine (LIFE TECHNOLOGIES). The choline content of the media was approximately 100 μM. Cells were grown at 37 °C in a 5% CO_2 humidified atmosphere. Growing cultures of CHO cells at approximately 50% confluence were exposed to DIPA, ^{14}C-DIPA, ^{14}C-DEA, ^{33}P-phosphoric acid or ^{3}H-choline in medium or distilled water. Where appropriate, the pH of the medium was adjusted.

Concentrations of DIPA and ^{14}C-DIPA utilized were based upon the results of the initial cytotoxicity screen. Concentrations of ^{14}C-DEA (positive control), ^{3}H-choline and ^{33}P-phosphoric acid utilized were based upon published findings by Lehman-McKeeman and Gamsky (1999). Dose solutions were prepared by thorough mixing of a weighed amount of DIPA with a weighed amount of culture medium, adjusting the pH to 7.4 and subsequently diluting an appropriate volume in fortified culture medium. Dosing solutions containing ^{14}C-DIPA were prepared by adding 10 μCi ^{14}C-DIPA directly to each culture dish. Dosing solutions containing ^{33}P-phosphoric acid were prepared by adding 10 μCi ^{33}P-phosphoric acid directly to each culture dish. Dosing solutions containing ^{3}H-choline were prepared by adding 5 μCi ^{3}H-choline directly to each culture dish.

2.3. Cytotoxicity assay

CHO cells were seeded in 60 mm plastic culture dishes at a density of 3×10^5 in 3 mL of culture medium containing variable concentrations of neutralized DIPA dosing solution in triplicate. Upon incubation for 48 h, cells were rinsed with DPBS, trypsinized, pelleted by centrifugation at 2000*g* for 5 min and resuspended in DPBS. Live and dead cells were counted using a dye exclusion-hemocytometer method outlined by Strober (2003). No determination of the mode of toxicity was evaluated (e.g., apoptosis). Osmolality of separate samples of treated media were analyzed using a OSMETT-A Automatic Osmometer (Precision Systems, Inc.).

2.4. DIPA effects upon phospholipid synthesis

CHO cells were seeded at a density of 3×10^5 in 3 mL of culture medium in 60 mm plastic culture dishes and cultured overnight. A minimum of five cultures per treatment group was prepared. Neutralized DIPA at a non-toxic or a moderately toxic concentration and ^{33}P-phosphoric acid (10 μCi/plate) were then added directly to the culture dish and cultures were incubated for an additional 48 h. An uninnoculated dish, containing only medium and dosing material served as a blank control. Upon incubation, cells were washed with fresh medium, trypsinized, pelleted by centrifugation at 2000*g* for 5 min and resuspended and lysed in distilled water.

Preliminary to the evaluation of DIPA effects upon PL synthesis, an evaluation of the incorporation of DIPA into PtdEA, at several concentrations was also undertaken. CHO cells were seeded at a density of 3×10^5 in 3 mL of culture medium in 60 mm plastic culture dishs and cultured overnight. Neutralized ^{14}C-DIPA (10 μCi/plate) was then added in duplicate cultures at non-toxic (13 or 113 μg/mL) or moderately toxic (1013 μg/mL) concentrations, and cultures were incubated for an additional 48 h. An uninnoculated dish containing only medium and dosing material served as a blank control. No heat treated blanks were run as formations of PLs via spontaneous chemical events were unlikely. Upon incubation, treated cultures and the blank control were washed with fresh medium, trypsinized, pelleted by centrifugation at 2000*g* for 5 min and resuspended and lysed in distilled water. For comparative purposes duplicate cultures were treated with 512 μg/mL neutralized ^{14}C -DEA (8.2 μCi/plate).

2.5. Lipid extraction and analysis

Total lipids were extracted from lysed cells and blank controls using the method originally outlined by Bligh and Dyer (1959). Recovered lipids along with authentic standards of phosphatidylethanolamine (PtdEA), phosphatidylcholine (PtdCho), and sphingomyelin (SM) were then spotted onto Silica G coated thin-layer-chromatography (TLC) plates that had been prewashed with acetone, dried at 100 °C and stored desiccated until used. Lipids were separated using a chloroform:methanol:ammonium hydroxide (65:25:4) developing solution (Glaser et al., 1974). Standards were visualized using an appropriate spray reagent (Dittmer and Lester, 1964). Rates of PL synthesis and the metabolic incorporation of DIPA into PtdEA were quantitated from appropriate cultures by measuring ^{33}P and ^{14}C incorporation, respectively, using quantitative autoradiographic scanning of developed plates (Packard Instant Imager, Downers Grove, IL). Measurements of the % of a particular PL were calculated by comparing the scanned ^{33}P CPMs for a specific PL and the total CPMs recovered for the specific chromatogram (i.e., the total "lane") upon correction for control areas of the TLC plate. The total amount of ^{33}P radiolabel recovered in extracted PLs was determined via liquid scintillation counting of an aliquot of each extract; however, a direct comparison of applied radioactivity and recovered radioactivity is not possible due to the marked differences in counting efficiency of scanning and liquid scintillation technologies employed. Migration patterns for radiolabeled spots were compared to those of known standards which were used as the sole determinant of PL species. ^{14}C and ^{33}P radioactivity in samples were converted to weight equivalents of DIPA and appropriate PLs based upon the specific activity of administered ^{14}C-DIPA and ^{33}P-phosphoric acid.

2.6. DIPA effects upon choline uptake

CHO cells were seeded at a density of 2×10^5/well in 1 mL of culture medium in 24-well culture plates, four cultures per group, and cultured