

The Metabolism of the Pancreas Carcinogen N-nitrosobis(2-oxopropyl)Amine by Hamster Pancreas Duct Epithelial Cell Clones; Evidence for Different Metabolic Efficiencies and Response to Cytochrome P450 Inducers

Carol Kolar, Terence Lawson

Eppley Institute for Research in Cancer and Allied Diseases
University of Nebraska Medical Center
Omaha, Nebraska, USA

ABSTRACT

Context We have isolated five stable clones from a primary culture of Syrian golden hamster pancreatic duct epithelial cells and have designated them as CK1 through CK5.

Design Here we describe the ability of two of these, CK1 and CK5, to metabolize the pancreas carcinogen N-nitrosobis(2-oxopropyl)amine. The metabolism was assessed as the production of mutated V79 cells in a CK cell/V79 co-culture set up.

Results At a dose of 0.1 mM N-nitrosobis(2-oxopropyl)amine, the CK1 cells produced 82.3 ± 17.2 mutants/ 10^6 survivors while the CK5 cells produced only 33.2 ± 10.8 mutants/ 10^6 survivors, both are mean \pm SD (n = 8). Furthermore, both cell types responded differently to two inducers of cytochrome P450 activity, namely Arochlor 1254 and EtOH. Arochlor 1254 treatment did not affect the metabolizing ability of CK1 cells while EtOH treatment resulted in a twofold increase in the mutation frequency. Arochlor and EtOH treatment inhibited the ability of CK5 cells to metabolize N-nitrosobis(2-oxopropyl)amine.

Conclusions These data show that the duct epithelium of the pancreas is a multi-cellular tissue and the different cell types within the epithelium have different abilities to metabolize xenobiotic chemicals.

INTRODUCTION

N-nitrosobis(2-oxopropyl)amine (BOP) induces ductular adenocarcinomas in the pancreas of Syrian golden hamsters, the tumors arising from cells within the duct epithelial network [1]. BOP requires metabolism to be active, first to a α -hydroxy derivative which breaks down spontaneously to give a methylating cation [2]. If BOP acts like other dialkyl nitrosamines then the α -hydroxylation is mediated by one, or more, of the cytochrome P450 (CYP) family of enzymes [3]. At this time we do not know which of these CYP enzymes is responsible for this reaction. Previous studies have indicated roles for members of the CYP3A [4] and CYP4A [5] families in the metabolism of BOP and related nitrosamines. It is considered unlikely that there is one CYP enzyme that is responsible.

We have developed techniques for the isolation and maintenance *in vitro* of the epithelial cells of the pancreatic duct network [6] based on previous work by Githens *et al.* [7] and have used them in a series of studies on the action of BOP, and related nitrosamines and other carcinogens, using the V79 mutagenicity assay as the measure of metabolism [4,5,8]. In the present paper we describe the subdivision of a primary culture of pancreatic duct epithelial cells (HaDEC) into five separate clones, designated as CK1 through CK5, and present evidence that these are distinctly different. Specifically we present data on the ability of two of these clones, CK1 and CK5, to metabolize BOP to mutagenic species.

MATERIALS AND METHODS

Safety

BOP is a carcinogen in a variety of species and must be handled with extreme caution. Several of the agents used in the culture of the HaDEC and CK lines are of bovine or human origin and there is a finite, albeit small, risk of exposure to those agents responsible for the induction of bovine spongiform encephalitis or Creutzfeld-Jacob syndrome.

Cells

CK cells: CK cells are derived from pancreatic duct epithelial cells from a male Syrian golden hamster which had been treated with an ethionine/methionine promotion protocol after a single dose of BOP [9]. There was no evidence of neoplasia or any other lesion in the pancreas of this animal on histological examination. A detailed molecular biological analysis of the CK cells is shown below. The primary culture was set up according to the standard protocols established in our laboratory. When cysts of duct epithelial cells developed they were transferred to 60 mm collagen gel-coated dishes. From this point

they were subjected to different passage and culture protocols resulting in the five clones (CK1, 2, 3, 4, and 5). These cells were maintained, for four passages, in DME/F12 medium supplemented with appropriate additives (DME/F12⁺) (see below) and made to 20% (v/v) with conditioned medium from a parallel primary culture of hamster pancreatic duct epithelial cells. The first two passages were on tissue culture plastic, while the second two were on collagen coated dishes. At passage #5, the surviving cells were pooled in a 100 mm tissue culture dish for 2 hours to remove residual fibroblasts (which attach quickly), when the supernatant and epithelial cells were removed and divided into two aliquots for clonal selection.

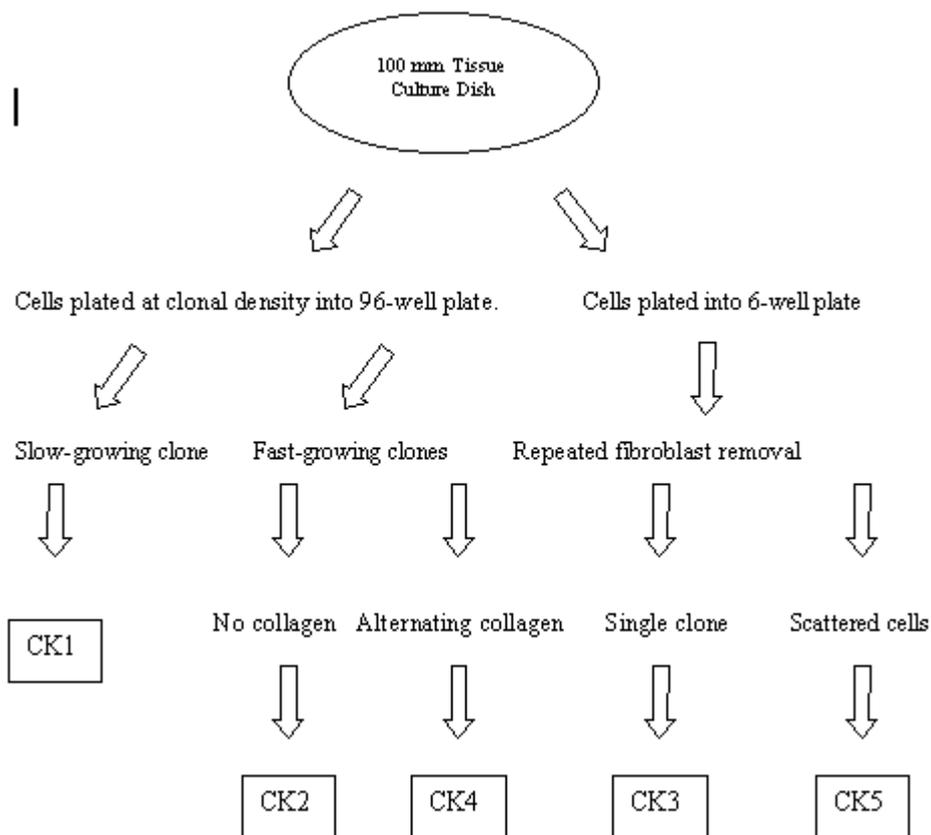
One aliquot was diluted to clonal density and plated in a 96-well tissue culture plate. Cells growing in this plate became CK1, CK2, and CK4. CK1 was a very slow growing clone in one well of the plate. CK2 and CK4 were clones derived from a pool of fast-growing cells from several wells. CK2 was the surviving clone from an aliquot of fast-growing cells plated onto tissue culture plastic in DME/F12⁺ medium with 20% conditioned medium for passages #6-10. CK4 was the surviving clone from fast-growing cells passaged alternately on tissue culture plastic and collagen gel coated plastic in passages #6 through 10 with 20% conditioned medium (as above).

The second aliquot was plated densely in a 6-well culture plate without collagen gel coating. Surviving cells were pooled and fibroblast removal was repeated as above, then cells were returned to plastic tissue culture flasks and passaged as needed for passages #6 through 10 in DME/F12⁺ medium with 20% normal conditioned medium. CK3 was a single surviving clone in one flask. CK5 was a pool of scattered surviving cells from another flask.

Beginning with passage #11, all lines were cultured in DME/F12⁺ medium, without the

addition of any conditioned medium, on plain tissue culture plastic. Lines exhibited doubling times of 36-50 hours soon after isolation, but gradually drifted to faster doubling times which stabilized around 24 hours by passage #30. Some lines have been passaged more than 50 times with no noticeable change in morphology or enzymatic activity.

This procedure is summarized in the following flow diagram.



The analyses to which the CK1 and CK5 cells used in this study cells were subjected are summarized in the following table. These analyses were performed by Dr. Bruce Rugerri (Personal communication).

Chemicals N-nitrosobis(2-oxopropyl)amine (BOP) was synthesized [12].

Table 1: Summary of diagnostic analyses performed on CK1 and CK5 cells

| | Tumorigenicity in nude mice [10] | Tumorigenicity in rat trachea model [11] | p16, p53 and RB expression | K-ras status |
|-----|----------------------------------|--|----------------------------|--------------|
| CK1 | - ve | - ve | Normal | Normal |
| CK5 | 0- ve | - ve | Normal | Mutated |

Mutagenicity

Mutagenicity was measured as resistance to 6-thioguanine according to the protocol of Jenssen [13]. CK cells were grown on filters (Schleicher and Shuell, Keene, NH) until they had reached confluence when they were co-cultured with the V79 cells. The filters were inverted so that the CK cells were in direct contact with the V79 cells. Co-culture took place over a sixteen-hour period. The CK cells were treated with arochlor (2 µg/ml) for 3 days prior to co-culture of the CK cells with the V79 cells. It was not present during the time when the co-culture was incubated with the mutagens. The CK cells were treated with EtOH (0.2 %; v/v) for 3 days prior to co-culture with the V79 cells and was present during the incubation with the mutagens.

ETHICS

The hamsters used to provide HaDEC, from which the CK1 and CK5 cells were derived, were housed under appropriate conditions that had been approved for such use by the U.S. Department of Agriculture and the American Association for Accreditation of Laboratory Animal Care (AAALAC). The use of these animals was approved by the UNMC Institutional Animal Care and Use Committee (IACUC).

STATISTICS

Data were analyzed by unpaired Student's t test [14].

RESULTS

The mutagenicity of BOP in V79 cells when activated by CK1 and CK5 cells is shown in table 2.

Table 2: Mutagenicity in V79 cells of BOP (0.1 mM) when activated by CK1 and CK5 cells

| | Mutants/ 10 ⁶ survivors ^a | | |
|-----------|---|--------------------------|---------------------------|
| | Untreated | Arochlor treated | EtOH treated |
| CK1 cells | 82.3 ± 17.2 | 76.7 ± 24.7 ^b | 161.2 ± 21.3 ^c |
| CK5 cells | 33.2 ± 10.8 ^d | 15.2 ± 9.9 ^e | 11.3 ± 8.2 ^f |
| HaDEC | 57.6 ± 14.1 | | 17.7 ± 6.4 ^g |

^a Mean ± SD, n = 8

^b Not statistically different from the mutation frequency of untreated CK1 cells (p 0.6)

^c Statistically different from the mutation frequency of untreated CK1 cells (p < 0.001)

^d Statistically different from the mutation frequency of untreated CK1 cells (p < 0.001)

^e Statistically different from the mutation frequency of untreated CK5 cells (p < 0.005)

^f Statistically different from the mutation frequency of untreated CK5 cells (p < 0.001)

^g Statistically different from the mutation frequency of untreated HaDEC (p < 0.001)

These data show that the CK1 cells are much more efficient at metabolizing BOP to mutagenic species than are the CK5 cells or the primary HaDEC from which both CK1 and CK5 cells are derived. Treatment of the CK1 cells with arochlor 1254 prior to the incubation with BOP did not affect the mutagenicity of BOP whereas treatment with EtOH prior to incubation increased the mutagenicity twofold. Treatment of CK5 cells with either arochlor 1254 or EtOH prior to incubation with BOP reduced the mutagenicity by half. Similarly treatment of HaDEC with EtOH reduced BOP mutagenicity threefold. The other three cell lines, CK2, CK3, and CK4 were without metabolizing ability when BOP was the substrate.

DISCUSSION

BOP induces pancreatic ductular adenocarcinoma in Syrian hamsters sometimes after a single dose [1]. This is the only reliable animal model for this type of cancer. While there is some controversy about the cells of origin of these tumors [15] it now seems clear that most of these tumors do arise from existing epithelial cells of the duct network. In common with other dialkylnitrosamines BOP requires enzyme-mediated metabolism to be active [3] being converted to the -hydroxy derivative. -Hydroxy-BOP decomposes spontaneously *via* a series of intermediates to give a methylating carbonium ion [16]. The nature of the enzymes responsible for the -hydroxylation of BOP is not known although it is presumed that they are members of the CYP superfamily of enzymes. The present study was initiated to address the issue of the responsible CYP enzymes. In the course of culturing HaDEC we were able to separate the five cell lines (CK1 - CK5) mentioned here.

The data in table 2 show that these two cell lines have very different abilities to metabolize BOP and that they also respond differently to two inducers of CYP activity, arochlor 1254 and EtOH. Arochlor 1254 is a broad spectrum inducer of CYP isoforms [17] principally CYP1A1, 1A2 [18] while EtOH is more specific apparently only inducing the activity of CYP1A1 and CYP2E1 [19]. Of interest is that EtOH induces CYP1A2 activity in human and hamster pancreas epithelial cells [20]. These data suggest that CYP2E1 is not involved in BOP metabolism, although they do not indicate which CYP isoforms are involved.

The significance of these studies is that several distinct clones can be separated from the same culture of primary cells. At the point that the cloning process began the culture would have consisted of duct epithelial cells. It will prove valuable to identify these clones in another manner such as surface markers so that their spatial position within the pancreas can be determined. It would be valuable to see if there is any zonation as occurs in the liver [21] and with it functional and response differences.

Received February 21st, 2000 - Accepted March 9th, 2000

Key words Carcinogens; Epithelium; Metabolism; Mutagenesis; Nitrosamines; Pancreatic Ducts.

Abbreviations BOP: N-nitrosobis(2-oxopropyl)amine; CYP: cytochrome P450;

Footnotes Supported by pilot funds from a GI SPORE grant from NCI and a Cancer Laboratory Core Grant (CA36727) from NCI

Correspondence

Terence Lawson
Eppley Institute for Research in Cancer
986805 Nebraska Medical Center
Omaha Ne 68198-6805, USA
Phone.: (402) 559 7475
Fax: (402) 559 4651
E-mail: talawson@unmc.edu

REFERENCES

1. Pour P, Runge RM, Birt D, Gingell RG, Lawson T, Nagel D, et al. Current knowledge of pancreatic carcinogenesis and its relevance to the human disease. *Cancer* 1981; 47: 1573-87.
2. Lawson T, Nagel D, Rogers D. The activation of nitrosamines that are carcinogenic to the pancreas. *Int J Pancreatol* 1991; 10: 9-22.
3. Yang CS, Smith TJ. Mechanisms of nitrosamine bioactivation and carcinogenesis. *Adv Exp Med Biol* 1992; 387: 385-94.
4. Lawson T, Kolar C. Mutation of V79 cells by N-dialkyl nitrosamines after activation by hamster pancreas duct cells. *Mutat Res* 1992; 272:139-44. [AN 93024601]
5. Lawson T. Mutagenicity in V79 cells of N-nitrosobis(2-hydroxypropyl)amine and N-nitrosobis(2-oxopropyl)amine activated by tissues from hamsters fed low and high fat diets. *Carcinogenesis* 1992; 13: 223-6. [AN 92154782]
6. Heimann TG, Githens SR. Rat pancreatic duct explants cultured in serum free medium on a porous support coated with an extracellular matrix. *Pancreas* 1991; 6: 514-21.
7. Lawson T, Kolar C. Mutagenicity of heterocyclic amines when activated by pancreas tissue. *Mutat Res* 1994; 325:125-8. [AN 95089796]
8. Kolar C, Lawson T. Mutagenicity of carcinogenic nitrosamines when activated by hamster and human pancreatic duct epithelial cells. *Cancer Lett* 1997; 117: 149-54. [AN 98018384]
9. Amanuma T, Mizumoto K, Tsutsumi M, Tsujiuchi T, Kitazawa S, Hasegawa K, et al. Initiating activity of diethylnitrosamine in a rapid production model for pancreatic carcinomas in Syrian hamsters. *Jpn J Cancer Res* 1991; 82: 632-7.
10. Tsao M, Earp HS, Grisham JW. Gradation of carcinogen-induced capacity for anchorage-independent growth in cultured rat liver epithelial cells. *Cancer Res* 1985; 45: 4428-32.
11. Tsao M, Duguid WP. Establishment of propagable epithelial cell lines from normal adult rat pancreas. *Exp Cell Res* 1987; 168: 365-75.
12. Nagel D, Kupper R. Synthesis of ¹⁴C-labeled N-nitrosobis(2-oxopropyl) amine. *J Labeled Cpd Radiopharm* 1971; 18:1081-5.
13. Jenssen D. A quantitative test for mutagenicity in V79 Chinese hamster cells. In: Kilbey BJ, Legator M, Nichols W, Ramel C, eds. *Handbook of mutagenicity test procedures*. Amsterdam: Elsevier Science Publishers, 1984; 269-90.
14. Gad S, Weil CS. *Statistics and experimental design for toxicologists*. Caldwell NJ, USA: The Telford Press, 1986: 70-92.
15. Schmied B, Liu G, Moyer MP, Hernberg ISB, Sanger W, Batra S, et al. Induction of adenocarcinoma from hamster pancreatic islet cells treated with N-nitrosobis(2-oxopropyl)amine in vitro. *Carcinogenesis* 1999; 20: 317-24.
16. Nagel DL, Lewis R, Fisher M, Stansbury K, Stepan K, Lawson T. ?-Oxidized N-nitrosocarbamates as models for DNA alkylation by N-nitrosobis(2-oxopropyl)amine. *IARC Sci Publ* 1987; 84: 71-4.
17. Phillipson CE, Ioannides C, Parke DV. Metabolic activation of dimethylnitrosamine to mutagens: role of cytochromes P-450 and P-448. *Toxicol Lett* 1982; 11: 95-101.
18. Rendic S, Di Carlo FJ. Human cytochrome P450 enzymes: A status report summarizing their reactions, substrates, inducers, and Inhibitors. *Drug Metab Rev* 1992; 29: 413-580.
19. Kessova IG, Decarli LM, Lieber CS. Inducibility of cytochromes P-450E1 and P-450IA1 in the rat pancreas. *Alcohol Clin Exp Res* 1998; 22: 501-4.
20. Lawson T, Kolar C, Reyes T. Mutagenicity of 2-amino-1-methyl-6- phenylimidazo[4,5-b]pyridine (PhIP) when activated by hamster pancreatic duct epithelial cells: A chemopreventive role for glutathione. *Mutat Res* 1997; 375: 73-8.
21. Oinonen T, Lindros KO. Zonation of hepatic cytochrome P-450 expression and regulation. *Biochem J* 1998; 329: 17-35. [AN 98070203]