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分担研究報告書

胆管発がんにおける1,2-ジクロロプロパンの修飾作用に関する研究

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研究要旨

近年、日本の印刷工場において胆管がん多発事例が報告されており、本事例の疫学調査からその原因物質として1,2-ジクロロプロパン（1,2-DCP）が指摘されている。1,2-DCPの胆管発がん性についてはラットおよびマウスを用いた発がん性試験において陰性であることがすでに報告されているが、胆・膵管発がん高感受性動物であるハムスターを用いた試験系は未だ行われていない。そこで本研究では、ハムスター胆道系および膵管に対する1,2-DCPの発がん修飾作用について、ハムスター二段階発がんモデルを用いて検討を行った。雄性シリアンゴールデンハムスターにイニシエーション処置としてN-nitrosobis(2-oxopropyl) amine (BOP)を投与し、1週間の休薬期間後、15および17週間1,2-DCPを強制胃内投与した。病理組織学的解析の結果、ハムスターの肝内胆管および膵管において前がん病変および腫瘍性病変の発生頻度・発生数に有意な変化が認められなかった。

以上の結果から、1,2-DCPはハムスター肝内胆管および膵管に対して発がん促進作用を有さないことが明らかとなった。ヒトの職業性胆管がんの発生環境には複数物質のばく露が指摘されていることから、今後1,2-DCPとジクロロメタンなど他の有機溶剤との複合ばく露による発がん性を評価する必要があると考えられた。

A．研究目的

1,2-dichloropropane（1,2-DCP）はテトラクロロエチレンと四塩化炭素のような塩素で処理された有機化学物質の産生で広く使われる化学中間体である。1,2-DCPおよびdichloromethane（DCM）を含んでいる塗料ストライパーに対する職業被曝が大阪のオフセット印刷工場で胆管がんの著しい増加と関係していることが、本事例の疫学的研究によって明らかとなっている。上述の疫学的研究の調査結果に基づいて、IARCは1,2-DCPをGroup1（ヒトに対する発がん性が認められる）に、DCMをGroup2A（ヒトに対する発がん性がおそらくある）に再分類した。DCMのマウス吸入がん原性試験で肝細胞性腫瘍と肺腫瘍を誘発することが既に報告されているものの、マウス、ラットまたはハムスターなどの実験動物を用いた発がん性試験で胆管発がん性を有さないことが明らかとなっている。同様に、1,2-DCPは強制胃内投与による発がん性試験において、マウスの肝細胞がんを誘発し、ラット吸入がん原性検査では鼻腔腫瘍を誘発したが、マウスまたはラットの胆管に発がん性はみられなかった。しかしながら、膵・胆道系において高い感受性を示すハム

スターを用いた1,2-DCPの発がん性の検討は未だなされていないことから1,2-DCPのハムスター肝内胆管に対する発がん性について評価する必要がある。加えて、膵管は胆管と発生学的な共通点を持ち、またヒトにおける膵がんの中で、膵管上皮に由来する膵管腺がんが最も多く、がん化の過程として膵管上皮における異型増殖の進行によるものと考えられている。膵管腺がんはマウスおよびラットではほとんどみられないが、Pourらによって開発されたBOPを用いたハムスターの膵がん二段階発がんモデルによって発生する膵管腺がんは、ヒト膵管腺がんとその発生過程が類似していること、組織学的に類似しているという二点において非常に優れていることから、1,2-DCP投与による膵管上皮への影響も併せて評価できると考えられた。

本研究では、雄のシリアンハムスターを用いた、ハムスター二段階発がん性試験を行い、ハムスター胆管・膵管における1,2-DCPの発がん修飾作用について評価を行った。

B．研究方法

[材料]

1. 化学物質

本実験の投与物質として、1,2-DCPを使用し、投与物質の調整にはコーンオイルを用いた。また、胆道・膵発がん誘導物質としてBOP(DIMS医学研究所より提供)を使用し、調製には生理食塩水を用いた。

2. 実験動物

5週齢雄性シリアンゴールデンハムスター87匹を用いて、1週間の馴化飼育期間を設けた後に試験に供した。飼育期間中の飲料水は水道水とし、基礎飼料はMF pelletを与えた。なお、動物は室温23 ± 2℃、相対湿度50 ± 20%、明期12時間の照明条件で飼育した。また、紙の床敷を入れたプラスチック製ケージに、3匹に分けて飼育し、ケージおよびチップを週1回交換した。屠殺までの実験期間中は、体重、摂餌量、摂水量を週1回測定した。

[方法]

1. 二段階発がん性試験

6週齢雄性シリアンゴールデンハムスターに対し、実験開始第1,3,5,7日目にイニシエーション処置としてBOPを10 mg/kg b.w.の用量で皮下投与し、1週間休薬後、実験開始3週目から1,2-DCPを0, 62.5, 125 mg/kg b.w.の用量で直接胃内投与する二段階発がん修飾作用群を3群に分けた。また、BOP処置の溶媒対照として生理食塩液10 mg/kg b.w.を同様に皮下投与し、1週間の休薬後、実験開始3週目から1,2-DCPを0, 125 mg/kg b.w.の用量で直接胃内投与するDCP単独群および非投与群の2群、合計5群に分けて実験を行い、実験開始から17週後に先行して二段階発がん修飾作用群から各群9匹ずつ剖検を実施した。肝内胆管および膵管について病理組織学的解析を実施したところ、膵管腺腫の用量相関的な増加傾向がみられたことから、19週時に残りの動物について剖検を実施した。今回実験に用いたBOPの投与用量、投与回数、実験期間については、過去のBOP二段階発がん性試験報告に基づき、選択した。また、1,2-DCPの投与用量については、本研究のハムスターにおける毒性試験の結果から選

択した。剖検時には、イソフルランを用いた吸入麻酔下で安楽死措置を行い、肝臓・総胆管・膵臓を摘出した。摘出した組織については、肝臓のみ重量測定を行い、得られた臓器をそれぞれホルマリン溶液で 3 日間固定を行った。

2. 病理組織学および免疫組織化学的検索

肝臓の全葉（左側葉、左中葉、右側葉、右中葉、尾状葉）をそれぞれ約 3 切片ずつ切り出し、膵臓も同様に全葉（胃葉、脾葉、十二指腸葉）に分け切り出しを行った。これらのパラフィンブロックから連続切片を切り出し、HE 染色し、光学顕微鏡下で病理組織学的検索を行い、胆管、膵管上皮細胞および肝細胞への影響の確認を行った。

また、細胞増殖能を評価するために Ki67 の免疫組織化学的染色を実施し、その陽性細胞率について定量的評価を行った。

[統計学的解析]

統計学的解析は Statlight program (Yukms Co., Ltd., Tokyo, Japan) を用い、F 検定もしくは Bartlett 検定を用いて等分散性を評価した。2 群検定において、等分散性であった場合は Student's T 検定を、分散にばらつきがみられた場合は Welch's T 検定を用いて評価を行った。また、多群検定において、等分散であった場合は両側 Dunnett 検定を、分散にばらつきがみられた場合に両側 Steel 検定を用いて評価した。肝臓病変発生率については Fisher の正確確率検定(カイ二乗検定)で評価した。また、全ての平均値は Mean ± SD として表し、$P < 0.05$ 以下のものを統計学的に有意であるとみなした。

[倫理面への配慮]

大阪市立大学の動物飼育施設における動物実験取り扱い規約に基づき、動物を飼育した。屠殺は動物に苦痛を与えないために麻酔下にて実施した。

C．研究結果

1. 一般所見

剖検時における生存率、最終体重および肝重量、実験期間中における摂餌量および飲水量について Table.1 に示した。

BOP → 1,2-DCP 125 mg/kg b.w.投与群において、投与第 3 週より溶媒対照群との間に有意な体重減少が最終週までみられ、最終体重についても同様に有意な減少が認められた。また、二段階修飾作用群および 1,2-DCP 単独投与群ともに相対肝重量に有意な変化はみられなかった。

2. 肝内胆管および膵管における病理組織学的解析

肝内胆管における病理組織学的変化について Table.2 に、膵臓については Table.3 に、またそれぞれの所見における代表的な組織像について Figure.1 に示した。

肝内胆管における腫瘍性病変 (Figure.1-C) は BOP → 1,2-DCP 62.5 mg/kg b.w 投与群(19 週剖検群)においてみられたが、14 例中 1 例のみ (0.07 ± 0.27) であり、BOP → 1,2-DCP 125 mg/kg b.w. 投与群において 17、19 週ともに腫瘍性病変がみられなかったため、前がん病変である異型過形成(Figure.1-A,B)を INHAND に基づいて検索を行った。その結果、1,2-DCP 投与による肝内胆管の異型過形成の発生頻

度および発生数に有意な変化および用量相関性は17、19週ともにみられなかった。

また、膵臓における腫瘍性病変(Figure.1-E,F)は二段階修飾作用群にみられ、さらに17週剖検群で用量相関傾向がみられたが、19週剖検群における溶媒対照群において15匹中5例（0.33±0.49）、BOP → 1,2-DCP 62.5 mg/kg b.w. 投与群では14匹中4例（0.29 ± 0.46）、そしてBOP → 125 mg/kg b.w. 投与群では15匹中4例(0.27 ± 0.46) の腫瘍性病変が発生し、1,2-DCP投与による腫瘍性病変の発生頻度および発生数の増加がみられなかった。そのため、膵管上皮の異型過形成(Figure1-D)の発生頻度および発生数についてINHANDを基に検索を行った。その結果、1,2-DCP投与群および溶媒対照群間に有意な変化および用量相関性は17、19週ともに変化がみられなかった。また、1,2-DCP 125 mg/kg b.w.単独投与群および溶媒対照群間における異型過形成の発生頻度および発生数も同様に、有意な変化および用量相関性は17、19週ともにみられなかった。

3. 細胞増殖能の定量的解析

胆管上皮細胞および膵管上皮細胞における異型過形成(いずれも19週剖検群について)のKi-67陽性率の半定量的解析を行った結果、細胞増殖能に有意差はみられなかった。また、1,2-DCP125 mg/kg b.w.単独投与群および溶媒対照群の正常様胆管上皮および膵管上皮細胞においても同様に有意な差はみられなかった(Figure2-A,B)。さらに、膵管腺癌における細胞増殖能について検討した結果(17および19週剖検)、1,2-DCP投与による有意な変化はみられなかった(Figure.2-C)。

本研究は1,2-DCPのハムスター胆管および膵管に対する発がん修飾作用について検討するために、二段階発がん性試験を実施した。

病理組織学的解析の結果、BOP単独群と比較してBOP → 1,2-DCP 62.5および125 mg/kg b.w. 投与群において、腫瘍性病変の発生率および数について有意な変化は認められなかった。さらに、肝内胆管異型過形成における1,2-DCP投与による細胞増殖能についても有意な変化はみられず、さらにBOP投与の有無によらず、細胞増殖能に差がみられなかったことから、1,2-DCPがハムスター肝内胆管に対して発がん促進作用を有さないことが明らかとなった。

また膵管においても、17週に、BOP → 1,2-DCP125 mg/kg b.w.群で膵がんの増加傾向はみられたが、19週に膵がんの頻度および数に差はみられず、さらに17および19週の合計においても差はみられなかったこと。またBOP投与の有無によらず、異型過形成および膵管腺癌における細胞増殖能に差はみられなかったことから、1,2-DCPがハムスター膵管に対して発がん促進作用を有さないことも併せて明らかとなった。

これまでラット、マウス、ハムスターを用いた動物実験モデルにおいて、DCMおよび1,2-DCPの単独投与による胆管発がん性は認められていない。ヒトの職業性胆管がんの発生環境には複数物質のばく露が指摘されていることから、今後1,2-DCPとDCMあるいは他の有機溶剤との複合ばく露による発がん性を評価する必要がある。

本研究によって、1,2-DCP のリスク評価に対する重要な知見の提供に貢献することができた。

E. 研究発表

1. 論文発表

1) Wei M, Fujioka M, Yamano S, Shimomura E, Ishii N, Kakehashi A, Takeshita M, Wanibuchi H. Determination of Hepatotoxicity and Its Underlying Metabolic Basis of 1,2-dichloropropane in Male Syrian Hamsters and B6C3F1 Mice. Toxicol Sci, 2015 (in press).

2.学会発表

1) 下村衣里、魏　民、藤岡正喜、山野荘太郎、梯アンナ、三島胡桃、鰐渕英機、ハムスターBOP 二段階胆膵管発がんモデルを用いた 1,2-dichloropropane の発がん修飾作用の検討. 第 29 回発癌病理研究会, 9 月 1～3 日、いわき, 2014.
2) 魏　民、下村衣里、藤岡正喜、山野荘太郎、梯アンナ、石井真美、武下正憲、房　赫、鰐渕英機、ハムスター化学発がんモデルを用いた 1,2-dichloropropane の発がん修飾作用の検討. 第 73 回日本癌学会学術総会, 9 月 25～27 日, 横浜, 2014.
3) 下村衣里、魏　民、藤岡正喜、山野荘太郎、梯アンナ、鰐渕英機、ハムスターBOP 二段階膵胆管発がんモデルを用いた 1,2-dichloropropane(1,2-DCP) の発がん修飾作用の検討. 第 31 回日本毒性病理学会学術総会及び学術集会, 1 月 29～30 日, 東京, 2015.
4) 下村衣里、魏　民、藤岡正喜、山野荘太郎、梯アンナ、鰐渕英機、ハムスターBOP 二段階膵胆管発がんモデルを用いた 1,2-dichloropropane(1,2-DCP) の発がん修飾作用の検討. 第 31 回日本毒性病理学会学術総会及び学術集会, 1 月 29～30 日, 東京, 2015.
5) 下村衣里、魏　民、藤岡正喜、山野荘太郎、梯アンナ、鰐渕英機、1,2-DCP 投与によるハムスターおよびマウスの肝毒性メカニズムの検討. 第 14 回分子予防環境医学研究会, 2 月 13～14 日, 大阪, 2015

F. 知的所有権の取得状況

1. 特許取得

なし

2. 実用新案登録

なし

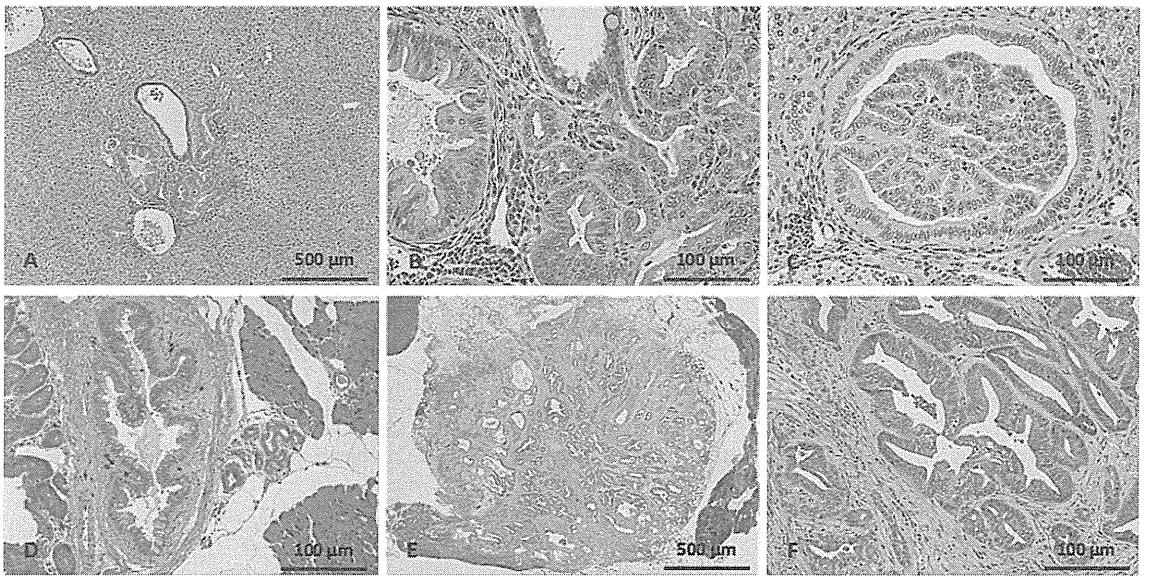

Figure 1
肝内胆管、膵臓における前がん病変および腫瘍性病変(第 19 週剖検群).
A: BOP→125mg/kg b.w. 1,2-DCP 投与群における肝内胆管異型過形成; B: A の強拡大像; C: BOP→62.5mg/kg b.w.1,2-DCP 投与群における胆管腺腫; D: BOP→125mg/kg b.w. 1,2-DCP 投与群における膵管上皮の異型過形成; E: BOP 単独投与群における膵管腺癌; F: E の強拡大像.

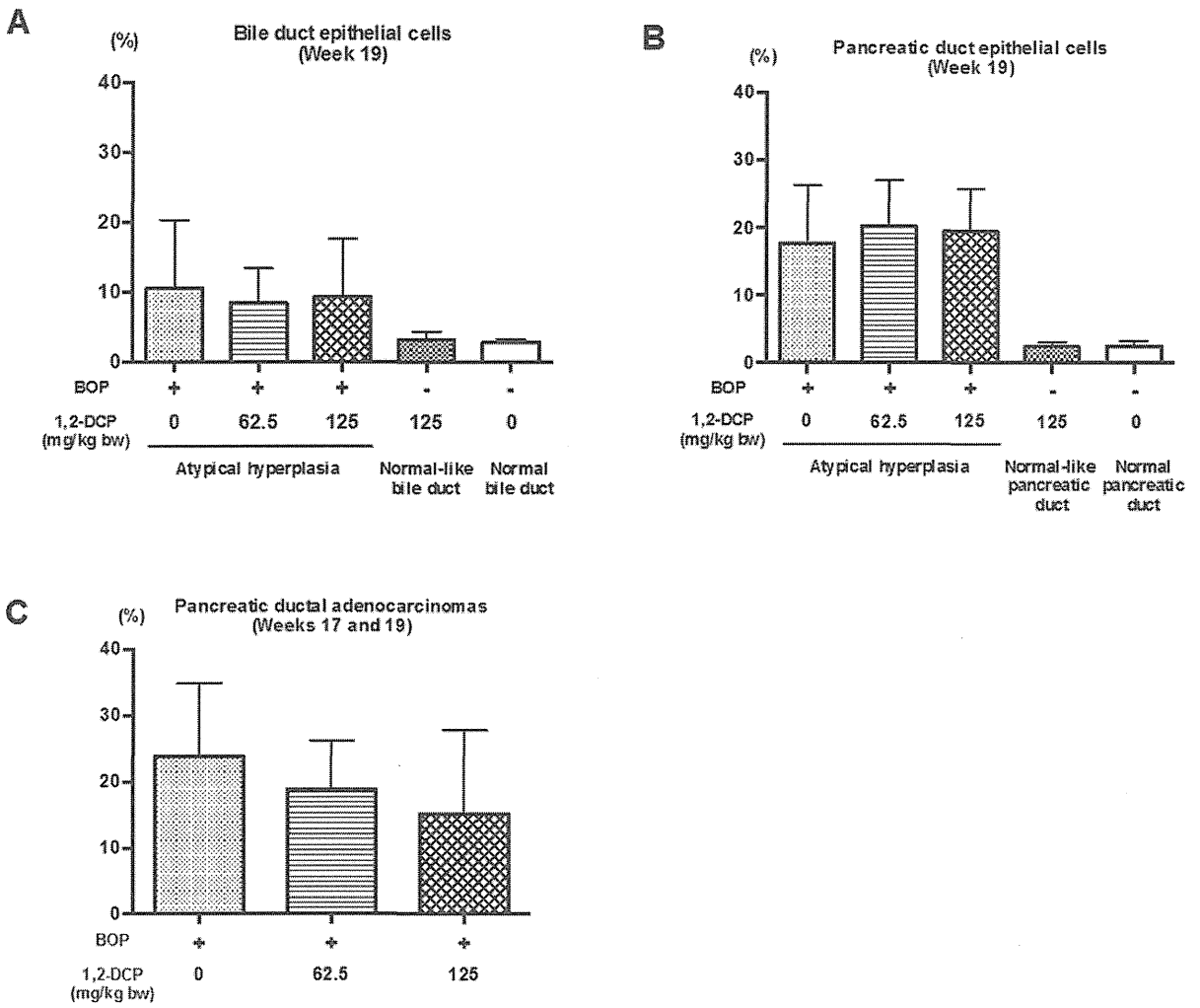

Figure 2

肝内胆管上皮細胞、膵管上皮細胞および膵管腺腫における Ki-67 陽性率の定量的解析

A:胆管上皮細胞における Ki67 陽性率(第 19 週剖検群); B: 膵管上皮細胞における Ki67 陽性率(第 19 週剖検群); C: 膵管腺癌における Ki67 陽性率(第 17 週および 19 週の総和)

Table.1 生存率、最終体重、肝重量、飲水量および摂餌量

	BOP initiation	1,2-DCP (mg/kg b.w.)	Effective no. of animals	Final body weight (g)	Absolute liver weight (g)	Relative liver weight (%)	Average water consumption (g/day/animal)	Average food consumption (g/day/animal)
Week 17								
1	+	0	9	168 ± 14	N.D.	N.D.	8.1	7.3
2	+	62.5	9	173 ± 11	N.D.	N.D.	8.1	7.3
3	+	125	9	146 ± 12*	N.D.	N.D.	7.5	6.7
Week 19								
1	+	0	15	170 ± 9	8.1 ± 0.7	4.8 ± 0.3	7.8	7.3
2	+	62.5	14[a]	173 ± 12	8.2 ± 1.1	4.7 ± 0.4	8.1	7.4
3	+	125	15	155 ± 12*	7.4 ± 0.8	4.7 ± 0.3	7.8	6.8
4	-	125	9	166 ± 14	7.7 ± 0.9	4.6 ± 0.2	7.5	7.4
5	-	0	6	181 ± 13	8.5 ± 1.1	4.7 ± 0.4	7.8	7.6

N.D. not determined

[a] 第12週時に1匹死亡、集計に含めない

* 有意差有り ($p<0.01$、vs. BOP単独投与群)

Table.2 肝内胆管上皮における前がん病変および腫瘍性病変

	BOP initiation	1,2-DCP (mg/kg b.w.)	Effective no. of animals	Atypical biliary hyperplasia		Cholangioma	
				Incidence (%)	Multiplicity (No./hamster)	Incidence (%)	Multiplicity (No./hamster)
Week 17							
1	+	0	9	2 (22%)	0.44 ± 0.88	0	0
2	+	62.5	9	3 (33%)	0.78 ± 1.20	0	0
3	+	125	9	1 (11%)	0.11 ± 0.33	0	0
Week 19							
1	+	0	15	7 (47%)	0.80 ± 0.94	0	0
2	+	62.5	14[a]	6 (43%)	0.93 ± 1.44	1 (7.1%)	0.07±0.27
3	+	125	15	8 (53%)	0.73 ± 1.02	0	0
4	-	125	9	0	0	0	0
5	-	0	6	0	0	0	0

a 第12週時に1匹死亡、集計に含めない

Table.3 膵管における前がん病変および腫瘍性病変

	BOP initiation	1,2-DCP (mg/kg b.w.)	Effective no. of animals	Atypical ductal hyperplasia		Pancreatic ductal carcinoma	
				Incidence (%)	Multiplicity (No./hamster)	Incidence (%)	Multiplicity (No./hamster)
Week 17							
1	+	0	9	4 (44%)	0.77 ± 1.09	0	0
2	+	62.5	9	4 (44%)	0.67 ± 0.87	1 (11%)	0.11 ± 0.33
3	+	125	9	4 (44%)	0.44 ± 0.53	3 (33%)	0.44 ± 0.73
Week 19							
1	+	0	15	10 (67%)	1.33 ± 1.50	5 (33%)	0.33 ± 0.49
2	+	62.5	14[a]	8 (57%)	0.79 ± 0.89	4 (29%)	0.29 ± 0.46
3	+	125	15	8 (53%)	0.67 ± 0.82	4 (27%)	0.27 ± 0.46
4	-	125	9	0	0	0	0
5	-	0	6	0	0	0	0

a 第12週時に1匹死亡、集計に含めない

Ⅲ．研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Sobue T, Utada M, Makiuchi T, Ohno Y, Uehara S, Hayashi T, Sato K, Endo G.	Risk of bile duct cancer among printing workers exposed to 1,2-dichloropropane and/or dichloromethan	J Occup Health,		Epub ahead of print]	2015
Kubo S, Nakanuma Y, Takemura S, Sakata C, Urata Y, Nozawa A, Nishioka T, Kinoshita M, Hamano G, Terajima H, Tachiyama G, Matsumura Y, Yamada T, Tanaka H, Nakamori S, Arimoto A, Kawada N, Fujikawa M, Fujishima H, Sugawara Y, Tanaka S, Toyokawa H, Kuwae Y, Ohsawa M, Uehara S, Sato KK, Hayashi T, Endo G.	Case series of 17 patients with cholangiocarcinoma among young adult workers of a printing company in Japan.	J Hepatobiliary Pancreat Sci.	21(7)	479-88.	2014
Sato Y, Kubo S, Takemura S, Sugawara Y, Tanaka S, Fujikawa M, Arimoto A, Harada K, Sasaki M, Nakanuma Y.	Different carcinogenic process in cholangiocarcinoma cases epidemically developing among workers of a printing company in Japan	Int J Clin Exp Pathol.	7(8)	479-88	2014
Kubo S, Kinoshita M, Takemura S, Tanaka S, Shinkawa H, Nishioka T, Hamano G, Ito T, Abue M, Aoki M, Nakagawa K, Unno M, Hijioka S, Fujiyoshi T, Shimizu Y, Mizuguchi T, Shirabe K, Nishie A, Oda Y, Takenaka K, Kobarai T, Hisano T, Saiura A, Numao H, Toda M, Kuwae Y, Nakanuma Y, Endo G.	Characteristics of printing company workers newly diagnosed with occupational cholangiocarcinoma.	J Hepatobiliary Pancreat Sci.	21(11)	809-17	2014

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Tanaka S, Fukumoto N, Ohno K, Tanaka S, Ohsawa M, Yamamoto T, Nakanuma Y, Kubo S.	Cholangiocarcinomain a middle-aged patient working at a printing company.	Osaka City Med J.	60(1)	39-44.	2014
Kubo S, Takemura S, Sakata C, Urata Y, Nishioka T, Nozawa A, Kinoshita M, Hamano G, Nakanuma Y, Endo G.	Changes in laboratory test results and diagnostic imaging presentation before the detection of occupational cholangiocarcinoma.	J Occup Health	56(4)	317-22.	2014
Kubo S, Matsuzaki K, Seki T, Ohsawa M, Kumagai S, Endo G.	Severe acute hepatitis in a printing company worker: A case study.	J Occup Health	57:	87-90	2015;
Ito Y, Miyashiro I, Ito H, Hosono S, Chihara D, Nakata-Yamada K, Tsukuma H. et al.	Long-term survival and conditional survival of cancer patients in Japan using population-based cancer registry data.	Cancer Sci.	105:	1480-6.	2014
Yamada K, Kumagai S, Nagoya T, Endo G.	Chemical exposure levels in printing workers with cholangiocarcinoma.	J Occup Health.	56(5):	332-8.	2014
Wei M, Fujioka M, Yamano S, Shimomura E, Ishii N, Kakehashi A, Takeshita M, Wanibuchi H.	Determination of Hepatotoxicity and Its Underlying Metabolic Basis of 1,2-dichloropropane in Male Syrian Hamsters and B6C3F1 Mice.	Toxicol Sci,		in press	2015
Yamada K, Kumagai S, Endo G.	Chemical exposure levels in printing workers with cholangiocarcinoma. (Second report)	J Occup Health.	57	in press	2015
Takeuchi A, Ogawa Y, Endo Y, Kawai T, Namera A, Yamamuro K, Sumino K, Endo G.	Evaluation of urinary cyclohexanediols and cyclohexanol as biomarkers of occupational exposure to cyclohexane.	J Occup Health.	57	in press	2015
久保正二、竹村茂一、坂田親治、浦田順久、野沢彰紀、西岡孝芳、木下正彦、濱野玄弥、田中肖吾、菅原寧彦、中沼安二、圓藤吟史.	印刷労働者における胆管癌多発事例：新たな職業癌.	日本消化器病学会雑誌	111:	500-509	2014
久保正二.	胆管癌におけるトピックス:印刷事業場での胆管癌集中発生を含めて一.	胆膵の病態生理	30(1),	1-4	2014

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
久保正二、竹村茂一、坂田親治、田中肖吾、中沼安二、圓藤吟史.	印刷労働者に多発した胆管癌.	胆道	28	763-771	2014
中沼安二、角田優子、佐藤保則、久保正二.	職業的暴露（印刷業）による胆管・胆道癌の特徴：病理所見および発癌メカニズムを中心に.	肝胆膵	69	1079-1085	2014
虻江　誠、鈴木雅貴、塚本啓祐、青木　優、久保正二.	印刷会社勤務歴を有する肝内胆管癌の1例.	胆道	28:	696-702	2014
金子 麗奈, 中崎 奈都子, 田川 徹平, 大石 千歳, 原 浩二, 金 民日, 草柳 聡, 馬場 俊 之, 小川 正純, 佐藤 譲 .	若年性胆管癌の疫学的特徴について—職業性胆管癌調査の予備的解析—.	Nippon Shokakibyo Gakkai Zasshi	111(3)	510-511	2014
圓藤　吟史	職業がんのリスクとその対策.	病理と臨床	32(6)	656-661	2014.6
圓藤　吟史	今日の労働科学の課題－化学物質のリスクアセスメント.	労働の科学	69(8):	20-21	2014.8
圓藤　吟史	オフセット印刷と胆管がん－新たな職業性疾患の発見.	JIM	24(9)	826-827	2014.9
圓藤　吟史	新しい職業がん.	日本医事新報	(4743)	57,	2015.3
圓藤　吟史	新しい化学物質管理.	日本医事新報	(4747)	54	2015.4
圓藤　吟史	胆管がん　産業保健スタッフがいたら救えたか？	銀杏	43:	1-2	2015
中川　圭、片寄　友、石田和之、林　洋毅、森川孝則、吉田　寛、元井冬彦、内藤　剛、久保正二、海野倫明.	印刷業職業性胆管癌に対する化学放射線療法と根治的肝切除の経験.	日本消化器病学会雑誌		印刷中	

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ORIGINAL ARTICLE

Case series of 17 patients with cholangiocarcinoma among young adult workers of a printing company in Japan

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Abstract

Background An outbreak of cholangiocarcinoma occurred among workers in the offset color proof-printing department at a printing company in Japan. The aim of this study was to clarify the characteristics of the patients with cholangiocarcinoma.

Methods This was a retrospective study conducted in 13 Japanese hospitals between 1996 to 2013. The clinicopathological findings of cholangiocarcinoma developed in 17 of 111 former or current workers in the department were investigated. Most workers were relatively young.

Results The cholangiocarcinoma was diagnosed at 25–45 years old. They were exposed to chemicals, including dichloromethane and 1,2-dichloropropane. The serum γ-glutamyl transpeptidase activity was elevated in all patients. Dilated intrahepatic bile ducts without tumor-induced obstruction were observed in five patients. The cholangiocarcinomas arose from the large bile ducts. The precancerous or early cancerous lesions, such as biliary intraepithelial neoplasia and intraductal papillary neoplasm of the bile ducts, as well as non-specific bile duct injuries, such as fibrosis, were observed in various sites of the bile ducts in all eight patients for whom operative specimens were available.

Conclusions The present results showed that cholangiocarcinomas occurred at a high incidence in relatively young workers of a printing company, who were exposed to chemicals including chlorinated organic solvents.

Keywords Biliary intraepithelial neoplasia · Cholangiocarcinoma · Intraductal papillary neoplasm of the bile duct · Organic solvent · Printing company

Introduction

Cholangiocarcinoma, a relatively rare cancer, arises from the biliary epithelium of the liver (intrahepatic cholangiocarcinoma) or in the extrahepatic bile ducts (extrahepatic cholangiocarcinoma) [1]. The peak age of cholangiocarcinoma diagnosis is within the seventh decade of life, and the disease occurs slightly more frequently in men [2–6]. Cholangiocarcinoma incidence rates vary markedly throughout the world, which presumably reflects differences in local risk factors and genetics. Although the incidence in the United States is 1–2 cases per 100,000 individuals, the highest rates of cholangiocarcinoma occur in Northeast Thailand (96 per 100,000 men) [3, 7, 8].

Recently, Kumagai et al. reported 11 cases of cholangiocarcinoma developing in former or current workers in the offset color proof-printing department at a printing company in Osaka, Japan [9]. However, they did not survey all of the patients with cholangiocarcinoma in the company or investigate any clinicopathological characteristics of the patients. In this study, we describe the clinical findings, laboratory test results, diagnostic imaging results, pathological findings, treatments, and prognosis of all patients with cholangiocarcinoma who were former or current workers of the printing company because this information is necessary to publicize to detect cholangiocarcinoma as a possible occupational disease and to prevent subsequent occurrence. We also discuss the possible carcinogenic progression of cholangiocarcinoma.

Subjects and methods

The subjects were 17 men with cholangiocarcinoma who were former or current workers at an offset color proof-printing department at a printing company in Osaka, Japan (Table 1). Cholangiocarcinoma was diagnosed between November 1996 and November 2012. Various types of chemicals, including chlorinated organic solvents such as 1,1,1-trichloroethane, dichloromethane, and 1,2-dichloropropane were used to clean ink residues in this department (Table S1). The chemicals used in this department have been changed several times. 1,1,1-Trichloroethane was used until December 1992, dichloromethane was used until March 1996, and 1,2-dichloropropane was used until October 2006. Various types of inks have also been used. This department was estimated to have 111 former or current workers (88 men and 23 women) between 1981 and 2012. Most workers were relatively young; a few workers were more than 50 years old and they were not exposed to high concentration of chlorinated organic solvent. Ten of the 111 workers could not be followed up after the resignation from the company. The clinical findings, laboratory test results, diagnostic imaging results, pathological findings, treatments and prognosis of the 17 patients were investigated. The 17 patients were treated at 13 hospitals. The information concerning clinical findings, including history of alcohol intake and smoking, were obtained from the medical records of each hospital and/or interviews with the patients. The laboratory test results were obtained from the medical records. For diagnostic imaging, computed tomography (CT) and ultrasonography were performed in all patients and magnetic resonance imaging (MRI), including magnetic resonance cholangiopancreatography (MRCP), was performed in 15 patients. Direct cholangiograms were obtained by endoscopic retrograde cholangiopancreatography (ERCP) in nine patients and during percutaneous transhepatic biliary drainage (PTBD) in two patients. The films of diagnostic images were available in 13 patients, and the imaging reports in the medical records were reviewed in the remaining four patients because the films had been retired. For 16 of the 17 patients, the pathological diagnosis was made by pathologists in the individual hospitals using the

Table 1 Clinical findings in patients with cholangiocarcinoma

Patient no.	Clinical findings				Laboratory tests			Diagnostic imaging	Diagnosis of cholangiocarcinoma		Treatments and prognosis after diagnosis	
	Age/sex	Symptom or health examination	Alcohol abuse	Smoking	γ-GTP (IU/l)	CEA (ng/ml)	CA19-9 (U/ml)	Methods for clinical diagnosis	Location and type of cholangiocarcinoma	Stage by TNM classification[1)]	Treatments	Prognosis
1	34/M	Epigastralgia, back pain	No	Yes	830	35.9	15200	Cholangiography during PTBD	ICC, mass-forming	IVA	Extended rt. hepatectomy, resection of extrahepatic bile duct, chemotherapy	7 years, 3 months, Dead
2	34/M	Rt. hypochondralgia	No	Yes	785	38.0	114	US, CT	ICC, mass-forming	IVA	Rt. trisectionectomy	1 year, 2 months, Dead
3	29/M	Jaundice, appetite loss	No	Yes	264	2.0	505	MRI	ECC, papillary	IB	Resection of extrahepatic bile duct	1 year, 7 months, Dead
4	25/M	Liver dysfunction	Yes	Yes	1729	8832.0	30.5	US, CT	ICC, mass-forming	IVB	Chemotherapy	1 year, 7 months, Dead
5	35/M	Liver dysfunction	No	Yes	2457	1.6	119.2	US, CT	ECC, papillary	IVA	Rt. hepatectomy, resection of extrahepatic bile duct[c]	2 years, 3 months, Dead
6	45/M	Rt. hypochondralgia, jaundice	No	Yes	1570	199.4	216394	US, CT	ECC, nodular	IVB	Conservative treatment	1 year, Dead
7	40/M	Liver dysfunction	No	Yes	1049	1.0	34	US, CT	ICC, mass-forming ECC, papillary carcinoma of Papilla of Vater	IIA[b]	Rt. hepatectomy, pancreaticoduodenectomy	6 years, 5 months, Alive
8	38/M	Liver tumor	No	No	208	2.9	2288	US, CT	ICC, mass-forming	IVA	Segmentectomy 8, chemotherapy	5 years, 8 months, Alive
9	39/M	Epigastralgia, weight loss	No	Yes	1983	28.6	4	US, MRI	ICC, mass-forming	IVB	Chemotherapy	9 months, Dead
10	40/M	Liver dysfunction[a]	No	Yes	1037	5.5	446	MRI	ICC, intraductal growth	IVA	Lt. hepatectomy[c], radiation, chemotherapy	3 years, 2 months, Dead
11	31/M	Liver dysfunction	No	Yes	1196	5.1	1084	US, CT	ECC, papillary	IVA	Extended rt. hepatectomy[c], chemotherapy	3 years, 3 months, Alive
12	39/M	Liver dysfunction	No	No	486	5.4	20.6	ERCP	ICC, intraductal growth ECC, papillary	IVA[b]	Lt. hepatectomy, radiation, chemotherapy	3 years, Alive
13	44/M	Liver dysfunction, liver tumor	Yes	Yes	1890	17.7	7749	US, CT	ICC, mass-forming	IVB	Chemotherapy	9 months, Dead
14	37/M	Liver dysfunction	Yes	Yes	451	9.6	50.5	MRI	ECC, nodular	IVB	Chemotherapy	1 year, 3 months, Alive
15	39/M	Liver dysfunction	No	Yes	347	1.5	105	ERCP	ICC, intraductal growth	IVA	Lt. hepatectomy, segmentectomy 7, chemotherapy	1 year, 1 month, Alive
16	31/M	Liver dysfunction	No	No	75	2.1	501	US, CT	ICC, mass-forming	IVA	Rt. hepatectomy, resection of extrahepatic bile duct[c], chemotherapy	1 year, Alive
17	34/M	Liver dysfunction	No	No	205	5	54	US, CT	ICC, intraductal growth	IVA	Extended lt. hepatectomy	11 months, Alive

γ-GTP γ-glutamyl transpeptidase, *CA19-9* carbohydrate antigen 19-9, *CEA* carcinogembryonic antigen, *CT* computed tomography, *ECC* extrahepatic cholangiocarcinoma, *ERCP* endoscopic retrograde chopnagiopancreatography, *ICC* intrahepatic cholangiocarcinoma, *MRI* magnetic resonance imaging, *PTBD* percutaneous transhepatic biliary drainage, *US* ultrasonography

[a] Liver dysfunction was detected during treatment for paranasal sinusitis

[b] Most advanced stage

[c] Non-curative resection

The stage of the tumors in patients 7 and 12 could not be classified because the patients had intra and extrahepatic cholangiocarcinomas

operative specimens in 12 patients and the biopsy specimens in four patients. The pathology examination could not be performed in another patient (patient no. 6); the diagnosis of cholangiocarcinoma was made by diagnostic imaging in this patient. The clinical findings, laboratory test results, diagnostic imaging results, and clinical course were re-evaluated by the medical staff (S.K., S.T., C.S., Y.U., A.N., T.N., M.K., G.H.) of Osaka City University Hospital. The operative specimens were available from 8 of the 12 patients treated surgically and were examined further by Y.N.

The pathological findings were recorded and described according to the World Health Organization's classifications for intrahepatic and extrahepatic cholangiocarcinoma [1]. Intrahepatic cholangiocarcinoma was grossly classified as mass-forming, periductal infiltrating, or intraductal growth (IG). Extrahepatic cholangiocarcinoma was grossly classified as papillary, nodular, or diffuse infiltrating. Preneoplastic or early preinvasive neoplastic lesions of the biliary tree were classified as flat dysplastic epithelium (biliary intraepithelial neoplasia; BilIN) or grossly visible papillary types (intraductal papillary neoplasm of the bile duct; IPNB). BilIN lesions were histologically classified according to their cellular and structural features as BilIN-1 (mild atypia), BilIN-2 (moderate atypia), or BilIN-3 (severe atypia corresponding to *in situ* carcinoma). In this study, BilIN-2 and BilIN-3 lesions were mainly surveyed because whether BilIN-1 lesions contain some reactive hyperplastic changes remains controversial. The classification of BilIN is described in Table 2. Cases of invasive carcinoma associated with IPNB (invasive IPNB) were classified as the IG type of intrahepatic cholangiocarcinoma or papillary type of extrahepatic cholangiocarcinoma [1, 10–12]. Other pathological terms used in this study were characterized or defined as follows. "Chronic bile duct injury" was used as a collective term of duct injuries such as epithelial damages, fibrosis of duct wall and periductal tissue, and chronic inflammatory cell infiltration in various combinations. "Proliferative changes of bile ducts" were used for the bile ducts with non-neoplastic biliary epithelial proliferation. "Bile duct sclerosis" indicates fibrous thickening of duct wall with or without additional periductal fibrosis.

Table 2 Pathological findings in the operative specimens

Patient no.	Chronic bile duct injury	Proliferative changes in bile ducts	BilIN-2/3	IPNB/ invasive IPNB	Main tumor metastasis	Lymph node
2	+	+	+	+	MF, poorly	–
8	+	+	+	ND	MF, mode	+
10	+	+	+	+	IG, well	+
11	+	+	+	+	pap, well	–
12	+	+	+	+	IG, pap, well	–
15	+	+	+	+	IG, well	–
16	+	+	+	+	MF, mode	+
17	+	+	+	+	IG, well	–

ND not determined because of small noncancerous hepatic tissue

Chronic bile duct injury was a collective lesion of various injuries such as epithelial damages, fibrosis of duct wall and periductal tissue, and chronic inflammatory cell infiltration. Proliferative changes were characterized by non-neoplatic biliary epithelial proliferation. BilIN, biliary intraepithelial neoplasia; BilIN-1 lesions presented with mild atypical cellular and nuclear features such as nuclear membrane irregularities or nuclear enlargements with only minimal disturbances to cellular polarity. BilIN-2 had evident aberrant cellular and nuclear features not sufficient to suggest overt carcinoma and focal disturbances in cellular polarity. BilIN-3 presented with diffuse disturbances in cellular polarity with or without distinct atypical cellular and nuclear features that corresponded to carcinoma *in situ*. In this study, BilIN-2/3 lesions were surveyed. IPNB, intraductal neoplasm of the bile; MF, mass-forming type intrahepatic cholangiocarcinoma; IG, intraductal growth type intrahepatic cholangiocarcinoma; pap, papillary type extrahepatic cholangiocarcinoma; poorly, poorly differentiated adenocarcinoma; mode, moderately differentiated adenocarcinoma; well, well-differentiated adenoacrcinoma

This study was approved by the ethics committee of Osaka City University, and all of the subjects or their legally authorized representatives (for deceased patients) provided written informed consent. The multicenter occupational cholangiocarcinoma study group consisted of investigators in 13 hospitals (including four university hospitals) and two universities.

Results

Clinical findings

The age of the patients at cholangiocarcinoma diagnosis ranged from 25 to 45 years old (mean, 36 years old; Table 1). They started to work at the printing company when they were 18 to 28 years old. The cholangiocarcinoma was diagnosed in 10 current and seven former workers. The period from the start of employment until diagnosis of cholangiocarcinoma or resignation from the company, which was considered the period of exposure to chemicals prior to the diagnosis of cholangiocarcinoma, ranged from 6 years, 1 month to 19 years, 9 months (median: 11 years, 4 months; Fig. S1). The longest period between the end of the exposure and the detection of cholangiocarcinoma was 9 years, 7 months. Of the 17 patients, all were exposed to 1,2-dichloropropane, 11 were exposed to dichloromethane, and eight were exposed to 1,1,1-trichloroethane. The period of exposure to chlorinated organic solvent ranged from 6 years, 1 month to 16 years, 1 month (median: 9 years, 7 months).

Of the seven patients in whom carcinomas were detected after resignation from the company, two were employed at a different printing company that did not use 1,1,1-trichloroethane, dichloromethane, or 1,2-dichloropropane, and five were employed in jobs without usage of chemicals.

Of the 17 patients, five patients suffered from abdominal pain, jaundice, and/or appetite loss. Abnormal liver function test results or liver tumors were detected in 11 patients during health examinations. In another patient (no. 10), liver dysfunction was detected during treatment for paranasal sinusitis. Four patients were non-drinkers, three patients were habitual alcohol consumers (≥80 g of ethanol daily) [13], and 13 patients were smokers.

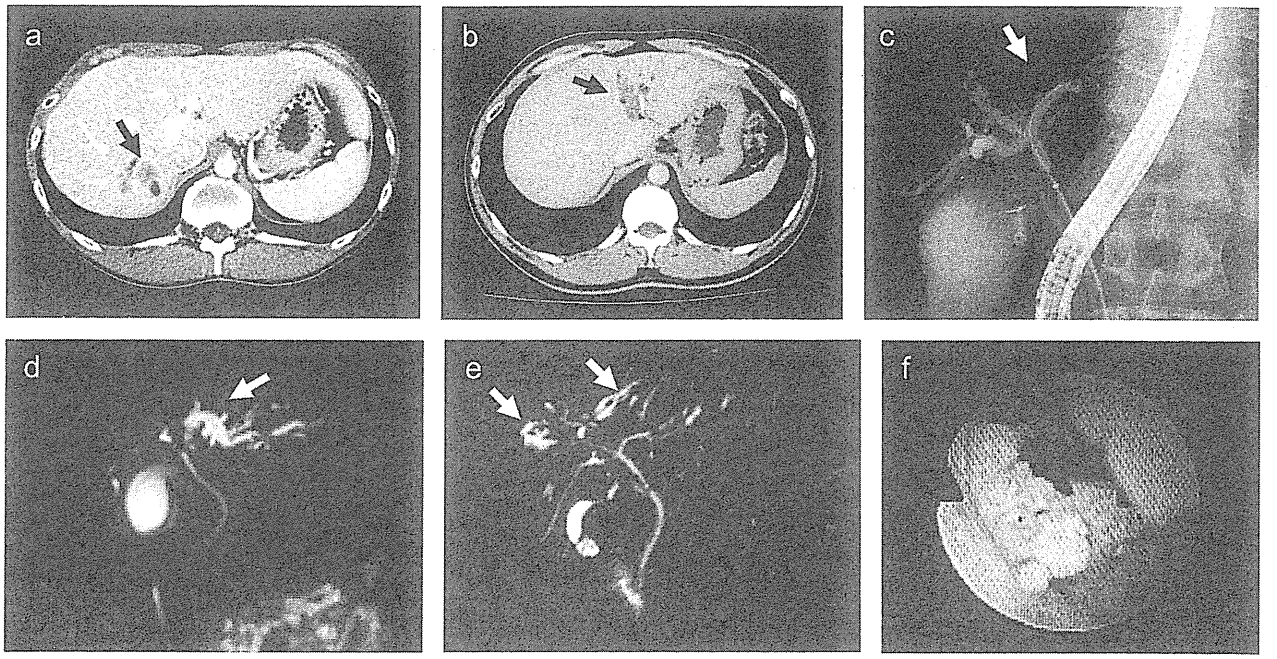

Fig. 1 Diagnostic imaging of patients with cholangiocarcinomas
(**a**) Intrahepatic cholangiocarcinoma of the mass-forming type (arrow), patient 16; (**b**) a dilated intrahepatic bile duct with a papillary tumor (intraductal growth type, arrow), patient 12; (**c**) stenosis of the intrahepatic bile duct due to cholangiocarcinoma (arrow), patient 15; (**d**) dilated intrahepatic bile ducts due to cholangiocarcinoma (arrow), patient 10; (**e**) dilated intrahepatic bile ducts without tumor-induced obstruction (arrow), patient 15; (**f**) intraoperative cholangiofiberscopy exhibiting lesions protruding into the bile duct, patient 15

Laboratory test results

At the time of cholangiocarcinoma diagnosis, the serum concentrations of total bilirubin were elevated in eight patients. Serum activities of aspartate and alanine aminotransferase were elevated in 13 patients and 14 patients, respectively. The serum γ-glutamyl transpeptidase (γ-GTP) activity was elevated in all of the patients (Table 1). In the five patients with available laboratory test results from several years prior to the diagnosis of cholangiocarcinoma, the serum γ-GTP activity was consistently high. The serum concentrations of carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA 19-9) were elevated in 10 and 13 patients, respectively. The tests for serum hepatitis B surface antigen and hepatitis C virus antibodies were negative in all of the patients. A test for the hepatitis B core antibody was positive in one of the 11 patients examined.

Diagnostic imaging

Clinical diagnosis by imaging was made first by ultrasonography, CT, MRI, ERCP, and/or cholangiograms during PTBD (Table 1). Space-occupying lesions were demonstrated in nine patients (no. 1, 2, 4, 6–9, 13, and 16) using CT, MRI, and/or ultrasonography (Fig. 1a). Bile ducts with papillary, villous, or protruding tumors were observed in five patients (no. 3, 5, 11, 12, and 17) on ultrasonography, CT, and/or ERCP (Fig. 1b). Stenosis or obstructions of the bile duct were observed in five patients (no. 1, 7, 10, 11, and 15; Fig. 1c) as observed on MRCP and/or direct cholangiograms. By contrast, the dilatation of peripheral bile ducts due to tumor-induced bile duct obstruction was observed in 11 patients (no. 1, 2, 4–7, and 9–14) in ultrasonography, CT and/or MRI (Fig. 1d). Dilated intrahepatic bile ducts without tumor-induced obstructions were observed in five patients (no. 8, 12, and 15–17) on ultrasonography, CT, and/or MRI (Fig. 1e). In these five patients, findings that were noted on cholangiography appeared to correspond to those of primary sclerosing cholangitis (PSC), including multiple intrahepatic bile duct strictures with or without fusiform dilatation [14]. Intraoperative cholangiofiberscopy, which was performed on three patients (no. 15–17), revealed epithelial irregularities of the bile ducts, IPNB, and papillary lesions protruding into the dilated bile ducts without tumor-induced obstruction (Fig. 1f).

Diagnosis of cholangiocarcinoma

Intrahepatic cholangiocarcinoma was identified in 10 patients, extrahepatic cholangiocarcinoma was identified in five patients, and both intrahepatic and extrahepatic cholangiocaricinomas were identified in two patients during

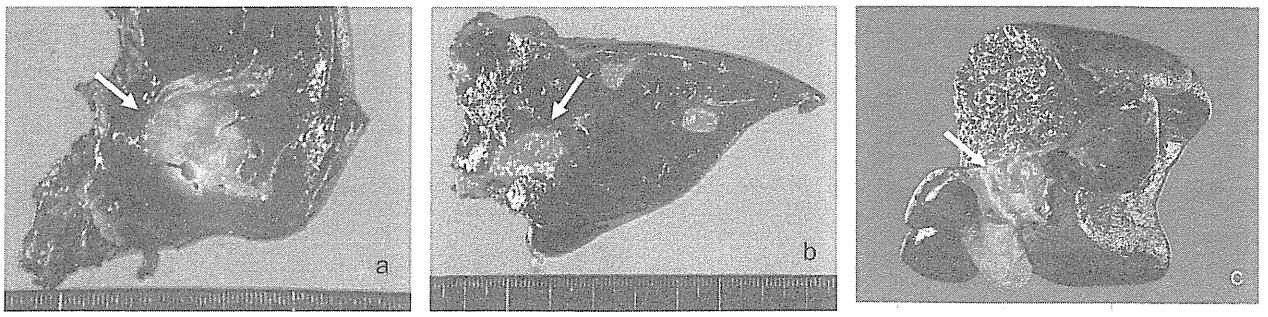

Fig. 2 Operative specimens
(**a**) intrahepatic cholangiocarcinoma of the mass-forming type (arrow), patient 16; (**b**) intrahepatic cholangiocarcinoma of the intraductal growth type (arrow), patient 15; (**c**) extrahepatic cholangiocarcinoma (hilar cholangiocarcinoma) of the papillary type (arrow), patient 11

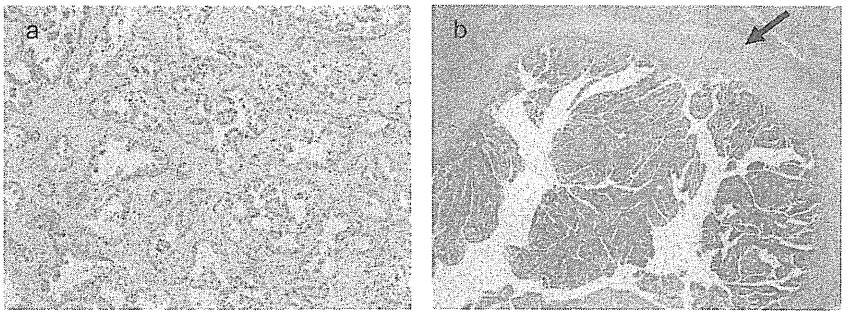

Fig. 3 Histological features of cholangiocarcinomas
(**a**) Intrahepatic cholangiocarcinoma of the mass-forming type with tubular adenocarcinoma, patient 8, hematoxylin and eosin (H-E) staining, 400 × magnification; (**b**) Intrahepatic cholangiocarcinoma of the intraductal growth type with focal invasion (arrow) (corresponding to intraductal papillary neoplasm of the bile duct with an associated invasive carcinoma), patient 12, H-E staining, 150 × magnification

diagnostic imaging and/or surgery (Table 1). Among the 12 patients with intrahepatic cholangiocarcinoma, the tumors were classified as mass-forming type in eight patients (Fig. 2a) and as IG type in four patients (Fig. 2b). Among the seven patients with extrahepatic cholangiocarcinoma, the tumors were classified as papillary-type cholangiocarcinoma (Fig. 2c) in five patients and as nodular-type in two patients. Surgically resected specimens or biopsy specimens revealed variably differentiated adenocarcinomas compatible with cholangiocarcinoma in all 16 patients in whom pathological examination was performed. Comprehensive imaging studies and gross analyses of these patients demonstrated that the main and most invasive cholangiocarcinoma lesions were located in the common hepatic duct, the left or right hepatic duct, or the first to third branches of the intrahepatic bile duct (also known as the large bile duct [15]).

Histopathological findings

The operative specimens were available from eight patients in this study (Table 2). Among the eight patients, three patients with mass-forming type of intrahepatic cholangiocarcinoma showed well-, moderate or poor adenocarcinoma (no. 2, 8, 16; Fig. 3a), and five patients with IG-type of intrahepatic cholangiocarcinoma and/or papillary-type extrahepatic cholangiocarcinoma exhibited well-differentiated papillary carcinoma (invasive IPNB) (no. 10–12, 15, 17; Fig. 3b). In the latter group, the invasive portions of the tumors were mucinous or tubular adenocarcinoma. Additionally, IPNB lesions without invasion were grossly and histologically detected in the various sites of the resected specimens from three patients (no. 2, 15, 16). Extensive intraductal spread of non-invasive neoplastic biliary epithelial cells, focally intraductal papillary pattern, was observed in three patients (no. 2, 15, 16). BilIN-1 and also BilIN-2/3 lesions were detected in various sites of the large intrahepatic bile ducts and/or hilar bile ducts and the peribiliary glands in all eight patients (Figs. 4a–c). Peribiliary glands, when they were identified in the specimens, showed hyperplastic changes and also atypical and preneopalstic lesions, corresponding to BilIN-2/3 lesions (Fig. 4d,e). In all eight patients, sclerosis of the bile duct with variable inflammatory cell proliferation, biliary epithelial injuries/focal bile duct loss, and biliary epithelial hyperplasia were also observed in various sites of

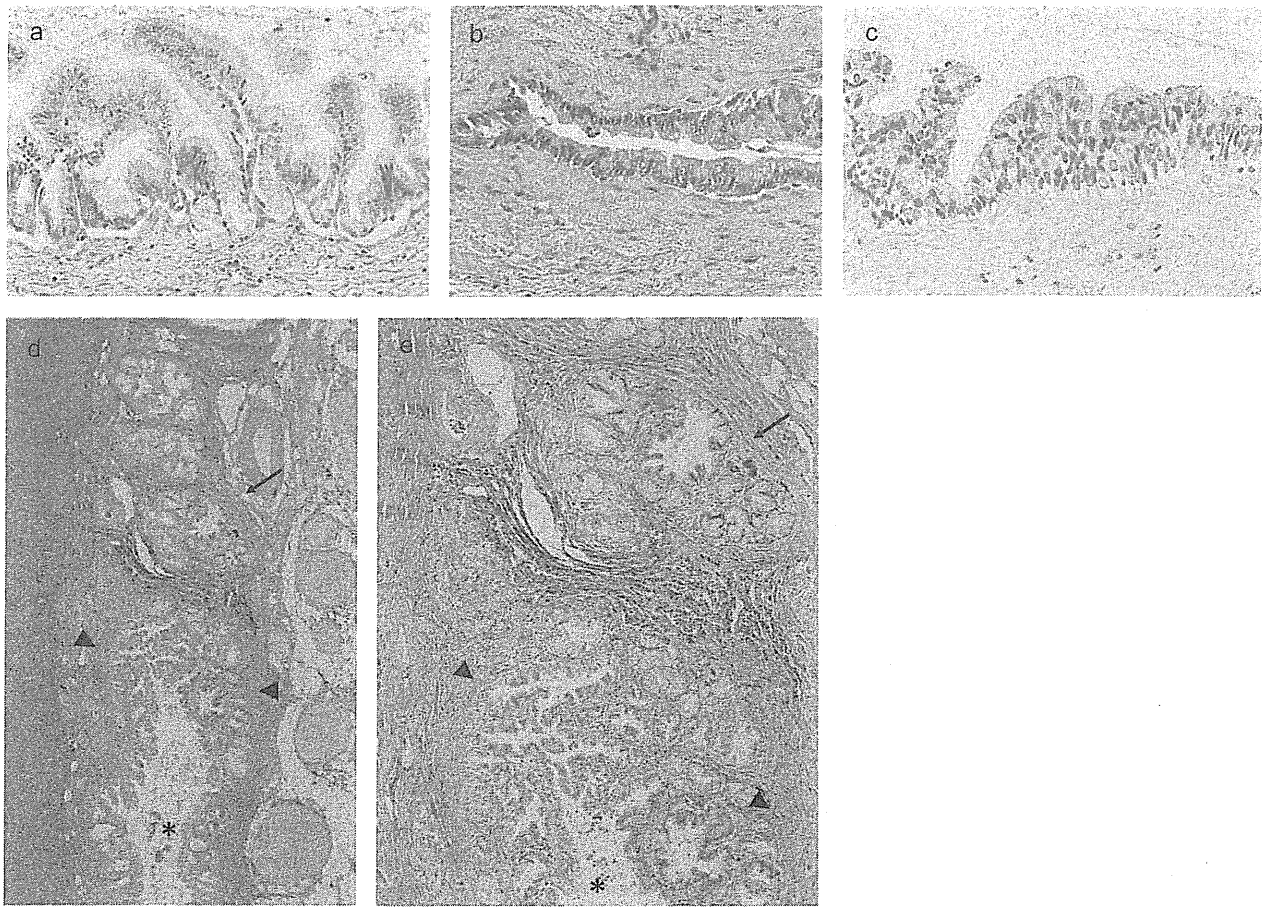

Fig. 4 Histological features of biliary intraepithelial neoplasia (BilIN) of bile ducts. (**a**) BilIN-1, patient 2. H-E staining, 250 × magnification. (**b**) BilIN-2, patient 12, H-E staining, 300 × magnification. (**c**) BilIN-3, patient 12, H-E staining, 300 × magnification. (**d**) Peribiliary glands of intrahepatic large bile duct (*) showing hyperplastic changes and foci of mild atypia, corresponding to BilIN-2 lesion, in the bile duct wall (arrow heads) and in the surrounding tissue (arrow), patient 10, H-E staining, 150 × magnification. (**e**) Higher magnification of D. H-E staining, 250 × magnification

the bile ducts in the noncancerous hepatic tissues (Fig. 5a,b). As for the pathology of the non-neoplastic, background liver, non-specific reactive changes or cholestatic changes secondary to obstruction or stenosis of bile ducts affected by cholangiocarcinoma were observed. Lymph node metastasis in the hepatoduodenal ligament and/or the along the common hepatic artery was found in three patients.

In summary, the precancerous or early cancerous lesions, such as BilIN and IPNB, as well as non-specific bile duct injuries, such as fibrosis, were observed in various sites of the bile ducts and peribiliary glands, particularly in the large and hilar bile ducts, in all eight patients. Invasive carcinoma was observed in the operative specimens.

No cirrhotic changes or other hepatobiliary diseases were detected in the noncancerous hepatic tissues of the eight patients. Thus, the laboratory test results, diagnostic imaging results, and/or pathological findings indicated that the 17 patients did not have any known risk factors for cholangiocarcinoma, such as PSC, hepatolithiasis, pancreaticobiliary maljunction, or liver fluke infection (*Clonorchis sinensis* and *Opisthorchis viverrini*) [2–4, 16–19].

Treatment and prognosis

Surgical resection was performed in 12 patients (Table 1). Curative resection could not be performed for 4 of these 12 patients because of the detection of cancer cells in the resected stumps of the bile ducts. In 10 of the 12 patients, dissection or sampling of the lymph nodes was performed. Four (no. 1, 8, 10, and 16) of the 10 patients exhibited metastases to the lymph nodes around the common bile duct or the common hepatic artery and/or peripancreatic lesions. Adjuvant chemotherapy with fluorouracil, gemcitabine, and/or S-1 (tegafur/gimeracil/oteracil potassium) was