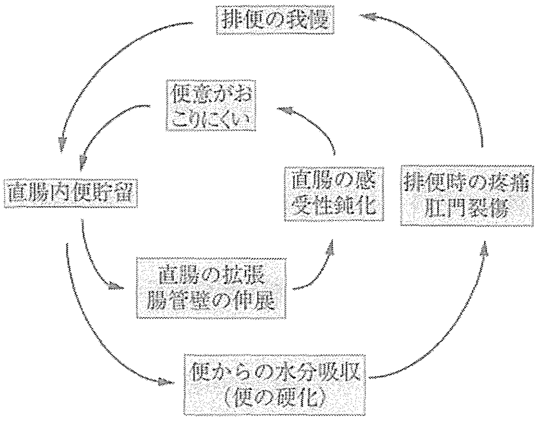

図1　便秘の悪循環

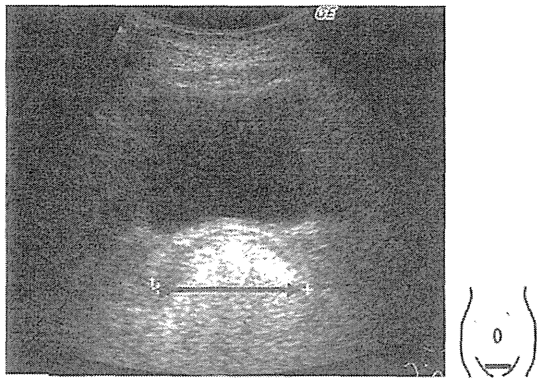

図3　慢性便秘児の直腸超音波所見
恥骨直上で水平にあてたプローブで得られる超音波断面において，直腸径が27（30）mm以上であれば直腸内に便塊が存在する可能性が高いとの報告がある

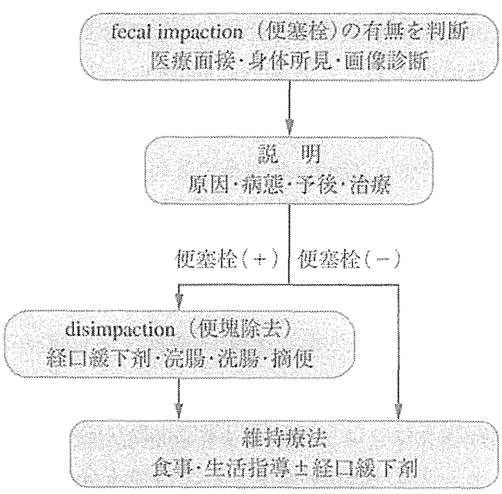

図2　慢性機能性便秘症の診断・治療手順

表2　fecal impaction（便塞栓）を強く疑わせる徴候

- 腹部触診で便塊を触知する例
- 画像上多量の便塊を認める例
- 力んでいるにもかかわらず排便に至らない例
- 5日以上十分量の排便が見られていない例
- 少量の軟便が1日に何回もパンツやオムツに漏れ出ている例（soiling：前述）
- ごく少量のころころ便が出ている例

禁＝fecal incontinence，soiling）ようになる．このような状態に陥った例，すなわち遺糞症を合併した例では，QOLが著しく低下し，また治療も著しく困難となる．そこで，その予備軍ともいえる，①排便時に肛門痛や出血が見られる例，②足をクロスするなどして肛門をしめ，排便を我慢する習慣のある例，③便失禁の見られる例，④画像上巨大結腸の疑われる例は，早期かつ積極的な治療が必要な例であり，それらの徴候の有無はつねに把握しておく必要がある．

また，後述するように，便秘の治療手順のうえでもっとも大切なことは，はじめに直腸内の便塊を完全にとりのぞき（disimpaction：便塊除去），その後，維持療法に入ることである[3)〜5)11)12)]（図2）．適切なdisimpactionを行わずに維持療法に入った場合，治療効果が得られにくいばかりではなく，便秘症がさらに悪化していく可能性が高いため，直腸内fecal impactionの有無を確認することはきわめて大切である．直腸内のimpactionの有無は，肛門指診で容易に診断されるが，肛門指診に恐怖感が強い例やくり返し評価が必要となる例では，超音波検査によっても診断可能であるとの報告もある（図3）．表2にあげるような徴候が一つでも認められる児では，直腸内が便塊で満たされている可能性が高いため，必ず指診などでimpactionが存在しないかを確認するか，impactionが存在するものとして治療する必要がある[6)]．

慢性便秘症の診療に際しては，患児および家族のQOLの低下に配慮し，患児や家族がどのような点に苦痛や困難を覚えているかを聞くことも大切である．たとえば，経肛門的治療に対する患児

の恐怖感，予後や治療の副作用に対する家族の不安感，遺糞症のある児では学校でのいじめや家族との葛藤などは，見逃されてはならない．

便秘治療の手順

慢性便秘の治療では，速やか，かつ完全に「便秘ではない状態」とし，それを維持し続けることが原則である[4]．

慢性便秘の治療は図2のフローチャートに従って行う．

①器質的疾患の疑われる例は，ただちにその診断が可能な施設に紹介する．

②機能性便秘では，はじめにfecal impaction（便塞栓）の有無が診断される必要がある．診断方法は，前述（診断の項）を参照のこと．

③impactionの有無にかかわらず，治療の第一段階は，患児・家族に便秘症の病態や治療を説明することである．便秘症の原因・悪化因子，悪循環のメカニズム，予後，治療の流れを説明し，長期にわたる適切な治療の必要性（しばしば年余に及ぶ）を強調することが必要である[3][12]．これによって患児や家族の不安を除くとともに，治療に対する先入観を是正することは治療成功のための重要なポイントである．遺糞症のある児では，その病態を説明するとともに，患児には失禁をコントロールできないこと，したがって患児や家族の落ち度ではないこと，便秘の治療により改善させることができることを十分に理解させる．

④impactionのある例では，まず，disimpaction（便塊除去）を行い，その後に維持療法（maintenance therapy＝再発予防治療）を開始する[3)～5)11)12]．fecal impactionのない例では，維持療法から開始する．

⑤維持療法は，疾患の説明，食事・生活・排便習慣指導，薬物治療を適切に組み合せて行う[2)3)6)～8]．

⑥治療開始後は，その効果を判定しつつ，長期間にわたって経過を見る必要がある．その際，排便日誌を記録させると効果の判定が正確に行えるのみならず，患児の励みになる効果も期待できる．適切に治療が行われても，1～2か月以内に「便秘でない状態」に至らない場合には，器質的疾患の有無あるいは治療法を再検討するため，器質的疾患の診断が可能で小児便秘症の治療に精通した医師・施設（専門家・施設）に紹介することが望ましい[1][6]．なお，そのような医師・施設の一部を紹介したホームページが公開されている（http://www.toilet.or.jp/health/counseling/：平成24年7月現在）．

disimpaction

前述のとおり，直腸内にimpactionのある児では，まず完全にdisimpactionが行われる必要がある．

disimpactionの方法には，経口の緩下剤投与と経肛門的治療（浣腸，坐薬，洗腸，摘便）がある．いずれも有効な手段であって一概に優劣をつけがたく，症例によって適宜に選択する必要がある[3][8]．

浣腸を行わなくても経口薬で十分な成功率が得られるとの報告もあり[13]，経口で十分にdisimpactionの目的を達することができる例では，それが望ましい．一方，浣腸は，患児がすでに苦しがっているなど比較的速やかに便塊除去が望まれる場合や，その場で効果を判断することが望ましい例では行われてよい治療の一つと考えられる．ただし，便秘症をもつ小児の多くは，過去のたび重なる排便時の肛門痛や，浣腸・摘便など経肛門的操作によるトラウマのために，肛門を診られる，あるいは触れられることに著しい恐怖感をもっている．そのような児に対して，無理矢理に浣腸を行うと，医師や病院に恐怖感をもつばかりでなく，ますます排便をいやがるようになる可能性が高い．また，極度に硬化した巨大な便塊が直腸内にある例に浣腸を行っても，排出は不可能で，患児が腹痛などで苦しむのみである場合があ

る．このような例では，むやみに浣腸を行うことにより，かえって便秘症を悪化させてしまうことがある．

摘便については，その適否についてコンセンサスが得られていない[3]．相当の痛みを伴う可能性があるためむやみに行われるべきではないが，便が著しく硬くなってしまっている例ではやむを得ない手段とも考えられる．

disimpaction目的の経口薬としては，欧米ではポリエチレングリコールが用いられることが多いが，わが国では維持療法と同様に，酸化マグネシウム（0.05 g/kg/日）がもっとも一般的であると思われる[6]．浣腸としては，わが国で頻用されるのはグリセリン浣腸である[6]．

disimpactionの実施期間は，3日以内，長くても1週間程度が目標となる．disimpactionが奏効すると便塞栓が消失し，以後は経口治療薬で良好な排便が維持される．遺糞症を伴う例では，便塞栓の消失と同時に便汚染も消失することが多い．経過がよければひき続いて維持療法を行う．

disimpactionがうまくいかない場合や，治療前に重度のfecal impactionを呈している場合など外来での治療が困難と判断された場合には，小児便秘の診断治療に精通した医師への紹介，入院治療，もしくは全身麻酔下摘便[5)14)]が考慮されるべきである．

維持治療

fecal impactionを認めない例や，disimpactionが完了した例では，以下に述べるような維持療法を開始する．

維持治療では，"すべての排便について痛みがなく排出できる"ように，継続的に便を硬くない状態に維持することが肝要である．いったん，便通が正常化した児でも，なんらかのきっかけで便が再び硬化して排便時に肛門痛を経験すると，すぐに悪循環（我慢ぐせ）に戻ってしまうことが多い．そのため，維持療法は患児が痛みを恐れず，安心して排便できる状態が確立されてからも，再発防止のため，年余にわたって続けられなければならない（3か月～3年に及ぶことが多い）[6]．また，そのことを治療初期の段階で家族に十分説明しておくことが望ましい．

維持治療は，生活・排便指導，食事指導，薬物療法によって行う．生活・排便指導，食事指導は全例で行われることが望ましい．ただし，その効果は限定的であり，薬物療法を加えることでより高い改善率が得られ，かつ速やかな改善が得られることが知られている．とくに，前述のように，①排便時に肛門痛や出血が見られる例，②足をクロスするなどして我慢し，肛門をしめる習慣のある例，③失禁の見られる例，④画像上巨大結腸の疑われる例，では治療が急がれるため，当初から積極的に薬物療法を加えるべきである[6]．しかし，すべての例で最初から薬物療法を加えるべきとは考えられず，軽症例では，①患者や家族への便秘の病態・予後・治療の説明と，②食事・生活・排便指導から開始し，1～2週間の経過で治療が奏効しない場合には，③薬物治療を加える，という手順でもよい[6]．

1．生活・排便指導

乳幼児では，ミルク・食事摂取量の不足がないかどうかをチェックし，それらを認めた場合には適宜是正する．幼児ではトイレトレーニングが便秘を悪化させたり，便秘の誘因になっていることがある．便秘症児のトイレトレーニングは便秘症治療により規則的な排便習慣が確立してから，保護者の精神的・時間的ゆとりのある時期を選んで行い，トレーニング中は失敗しても決して叱らないように指導する．排便しなくても5～10分座っていることができたら褒美としてシール，ぬり絵などを与えれば（賞賛でもよい），児の意欲を高めるのに役に立つとの意見もある．なお，綿棒による乳児の肛門刺激の有用性については，結論が得られていない[6]．

学童期以降の児については，便意を感じたとき

に我慢せずにトイレに行くように指導する．不規則な食習慣や日常生活と便秘の関係については，十分なエビデンスがあるとはいえないが，関係がないとしても是正することが望ましいことを配慮すべきである．ゆとりのある時間帯，とくに登校前にトイレに座る習慣をつける．ダイエット中の者には不適切なダイエットが行われていないかのチェックが必要である．

2．食事指導

便秘に対する食事療法の有効性については，さまざまな報告があり有効・無効とも結論が得られていない．しかし，安全性に問題がなく継続可能であり，一般的な健康維持について有利に働く可能性が高いため，まずすべての例で試みられるべきである[3)5)6)8)15)]．

もっとも効果が期待できるものは食物繊維である．食物繊維は，水溶性と不溶性のものに分類され，水溶性のものは大腸内で発酵・分解され腸内細菌叢に影響を与えることにより，不溶性のものは分解されることなく水分を吸収して膨潤化し便量を増加させることにより，効果を発揮すると推察されている[15)]．食物繊維の摂取により便通が改善するか否かについては異論があるが，明らかに摂取不足の例では，適当量を摂取することを勧めるべきであると思われる．

食物繊維の摂取の目的では，野菜（豆・根菜など），海藻，果物，穀類が適している．レタス，トマト，キュウリなどは比較的繊維含量が少ない[15)]．

スナック菓子や清涼飲料水の多くは，残渣が少ないうえ，カロリーが高く食事量を減らす可能性があるため便通にとって好ましくないと考えられる．ただし，その真偽に関するエビデンスは乏しい．

プロバイオティクスの有効性については結論が得られていないが，ヨーグルトの摂取により，便通が改善するとの意見がある[15)]．

脱水は便の硬化の原因となりうるため，夏期の過剰な発汗（寝汗など）には注意を要するが，脱水が認められない場合には，余分に水分を与えても便通は改善しない．したがって，授乳中の児に対して，便秘であるという理由で母乳やミルク以外に白湯などを与えることは好ましくない[3)]．

牛乳は比較的残渣が少ないため，離乳が遅れて栄養の多くをミルクや牛乳から摂取することが便秘の原因になるとの意見がある．また，牛乳アレルギーが便秘の原因になるとの報告がある[3)]．その真偽については意見が分かれるところであるが，通常の治療に反応しない場合は，2週間程度，牛乳摂取量の制限を試みることが勧められる．

3　薬物療法

生活・排便指導，食事指導のみで改善が十分でなければ，薬物療法の適応となる．初期から安易に薬物療法を行うことは必ずしも勧められないが，放置すれば便秘は悪循環をくり返すことは銘記されるべきである[6)]．

維持治療に用いられる薬剤として，浸透圧性下剤，刺激性下剤，消化管運動賦活薬などがある．わが国でおもに使用されている薬剤を表3に示す[6)]．原則として，塩類下剤や糖類下剤などの浸透圧性下剤から治療を開始する．これらの緩下作用は生理的であるため，習慣性がない，または少ない下剤と考えられている．いずれにせよ，服薬が便秘症の悪循環を放置するよりもはるかに予後を改善するということを十分に説明することが望ましく，コンプライアンスのアップに繋がる．また，効果発現には時間を要すること，また効果を上げるためには服用時に十分な水分を摂取する必要があることをあらかじめ伝えておくとよい．わが国でおもに使用されている塩類下剤は，酸化マグネシウムである[6)]．海外では使用頻度が高くなく，その効果については論文のエビデンスは不足しているが，標準的使用量では，糖類下剤に比して効果が確実であるとの見かたが多い．なお，2008年，厚生労働省から，酸化マグネシウム投

表3 便秘治療薬の種類

経口治療薬	浸透圧性下剤	塩類下剤	酸化マグネシウム（カマ）（酸化マグネシウム®，マグミット®）
			水酸化マグネシウム（ミルマグ®）
		糖類下剤	ラクツロース（モニラック®）
			マルトース（マルツエキス®）
	刺激性下剤	ピコスルファートNa（ラキソベロン®）	
		センノシド（プルゼニド®）	
	漢方薬		
	消化管運動賦活薬		
経直腸治療薬	浣腸薬	グリセリン（グリセリン浣腸液®）	
	坐薬	ビサコジル（テレミンソフト®坐薬）	
		炭酸水素ナトリウム＋無水リン酸二水素ナトリウム（新レシカルボン®坐薬）	

表4 わが国において選択頻度の高い薬剤と標準投与量

第一選択薬	乳児	糖類下剤	（例）モニラック®：0.33～1.3 g/kg（分3）
	幼児	糖類下剤	（例）モニラック®：0.33～1.3 g/kg（分3）
		酸化マグネシウム	（例）酸化マグネシウム®：0.05 g/kg（分1～2）
	学童	酸化マグネシウム	（例）酸化マグネシウム®：0.05 g/kg（分1～2）（成人量＝2 g/日）
第二選択薬	乳児	酸化マグネシウム	（例）酸化マグネシウム®：0.05 g/kg（分1～2）
		ピコスルファートNa	（例）ラキソベロン液®：3滴（7～12か月児）（分1）
	幼児	酸化マグネシウム	（例）酸化マグネシウム®：0.05 g/kg（分1～2）
		ピコスルファートNa	（例）ラキソベロン液®：6～7滴（1～6歳）（分1）
	学童	ピコスルファートNa	（例）ラキソベロン液®：10滴（7歳以上）（分1）
		センノシド	（例）プルゼニド®：〔成人量＝12～24 mg（1～2錠），分1就寝前〕

注：量については，適宜減可
　便秘でない状態を保ち，かつ下痢・腹痛をきたさない量に調節
　単剤で十分な効果が得られない場合には，2剤を併用することも可

与中に高マグネシウム血症をきたした15名（うち2名死亡）の報告および「長期投与例では血中マグネシウム濃度を測定するように」との勧告が出されたが，腎機能の正常な健常児で，常用量の投与では問題ない可能性が高い[16]．ただし，乳幼児で長期に使用する場合には，一度は血中濃度のチェックをすることが望ましい[16]．

緩下剤は，便が貯留してから大量に使用するよりも，毎日決まった量（週に3回以上自発排便があり，痛み・出血がおこらない量を，症例ごとに試行錯誤して決定する）を与えるほうがよい．学童の場合，はじめから大量に与えて学校で下痢になるようなことがあってはならず，休みの日などを利用する工夫も必要である．投与は長期にわたることが原則であるが，毎日のように排便がある状態を続けると，次第に緩下剤を減量できることが多い．慢性便秘例に対し第一選択薬，第二選択薬として使用されることの多い緩下剤を年齢別にあげる[6]（表4）．

なお，消化管運動賦活薬や漢方薬の慢性便秘症に対する効果については，十分なエビデンスがあるとはいえず，現時点では一般的な治療薬としては推奨できない．

薬剤のおもな副作用は，下痢，腹痛である．浸透圧性下剤や刺激性下剤の耐性や習慣性に関する明確なエビデンスはない．

4．外科治療

内科的治療に抵抗する例では，順行性浣腸路，

内肛門括約筋切開・部分切除，大腸部分切除などの外科治療が考慮されることがある．わが国においては，外科治療の対象となる機能性便秘症の例はきわめて少なく，限られた施設で行われているのが現状で，専門家と相談することが勧められる．

おわりに

慢性便秘症の治療においては，器質的疾患のある可能性や，患児・家族の苦痛，放置した場合の悪化傾向を考慮し，積極的な治療を長期に継続することが肝要である．また，多少とも診断・治療に困難を感じる例では積極的に専門家へ相談・紹介を行うべきであると考えられる．なお，本稿のもとになった専門家のコンセンサスは2～3年以内に診療ガイドラインとしてまとめられる予定である．

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著者連絡先
〒372-0812 群馬県伊勢崎市連取町3303-2
パルこどもクリニック
友政 剛

Journal of Pediatric Surgery (2013) **48**, 2373–2377

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Journal of
Pediatric
Surgery

www.elsevier.com/locate/jpedsurg

The most reliable early predictors of outcome in patients with biliary atresia after Kasai's operation

Taro Goda [a,*], Hisayoshi Kawahara [a], Akio Kubota [a], Katsuhisa Hirano [a], Satoshi Umeda [a], Gakuto Tani [a], Tomohiro Ishii [a], Yuko Tazuke [a], Akihiro Yoneda [a], Yuri Etani [b], Shinobu Ida [b]

[a]*Department of Pediatric Surgery, Osaka Medical Center and Research Institute for Maternal and Child Health, 840 Murodo-cho Izumi, Osaka, 594-1101, Japan*
[b]*Department of Pediatric Gastroenterology and Nutrition, Osaka Medical Center and Research Institute for Maternal and Child Health, 840 Murodo-cho Izumi, Osaka, 594-1101, Japan*

Received 14 August 2013; accepted 26 August 2013

Key words:
Predictor;
Prognosis;
Biliary atresia;
Direct bilirubin;
AST

Abstract

Background/Purpose: The purpose of this study was to determine reliable predictors of outcome of biliary atresia (BA) after Kasai's operation.

Patients and Methods: Fifty-four BA cases that underwent Kasai's operation at our institution over two decades were reviewed. The cases were divided into two groups: Group I: cases that required liver transplantation or died (n = 30) and Group II: cases alive with the native liver. Serum levels of total bilirubin (TB), direct bilirubin (DB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyltransferase (GGT) were measured sequentially after surgery. For cut-off determination, receiver operating characteristic (ROC) analysis was employed.

Results: Serum TB, DB, AST, and ALT in Group I were significantly higher than those in Group II at 1, 2, and 3 months after surgery ($p < .05$). The most reliable cut-offs determined by ROC analysis were DB of 0.7 mg/dl at 2 months (sensitivity; 93%, specificity; 75%) and AST of 94 IU/L at 2 months (sensitivity; 87%, specificity; 71%). The 54 cases were re-divided into three groups according to the cut-off values: group G (good) with DB and AST $<$ cut-offs (n = 16; Group I:II = 1:15), group M (moderate) with DB or AST $>$ cut-offs (n = 9; Group I:II = 4:5), and group P (poor) with DB and AST $\geq$ cut-offs (n = 29; Group I:II = 25:4). The 15-year survival rate in groups G, M, and P was 94%, 44%, and 22%, respectively ($p < .001$).

Conclusion: The combination of serum DB and AST at 2 months after Kasai's operation is a reliable predictor of long-term BA outcome.

* Corresponding author. Department of Pediatric Surgery, Osaka Medical Center and Research Institute for Maternal and Child Health, 840 Murodo-cho, Izumi, Osaka 594–1101, Japan. Tel.: +81 725 56 1220; fax: +81 725 56 5682.
E-mail address: tgoda@mch.pref.osaka.jp (T. Goda).

0022-3468/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jpedsurg.2013.08.009

Table 1 Preoperative characteristics of Groups I and II.

	Group I	Group II	*P* value
Kasai's operation (days)	58(24–101)	47 (7–120)	.13
Biliary obstruction (I:III) *	4:26	3:21	.75
TB (mg/dl)	10.1 (5.9–19.1)	10.2 (4.7–18.8)	.55
DB (mg/dl)	6.3 (3.4–13.9)	5.1 (1.8–12.6)	.07
AST (IU/L)	174(30–513)	134 (10–380)	.06
ALT (IU/L)	108 (10–226)	72 (8–323)	.15
GGT (IU/L)	369 (108–2290)	535 (99–1545)	.50

Data were shown as medians(ranges).
* BA classification of the Japanese Society of Pediatric Surgeons [4].

Table 2 Postoperative data of each group.

	Group I	Group II	*P* value
TB (mg/dl)			
1 month	5.8 (0.8–19.5)	1.9 (0.7–7.6)	<.001
2 months	5.4 (0.9–15.5)	0.8 (0.3–4.6)	<.001
3 months	4.0 (0.3–15.9)	0.8 (0.2–2.8)	<.001
DB (mg/dl)			
1 month	3.7 (0.3–13.5)	0.9 (0.2–4.9)	<.001
2 months	3.4 (0.3–11.2)	0.3 (0.1–2.3)	<.001
3 months	2.4 (0.2–10.5)	0.2 (0.1–2.0)	<.001
AST (IU/L)			
1 month	135 (59–389)	69 (49–186)	<.001
2 months	131 (58–306)	75 (36–168)	<.001
3 months	149 (29–338)	83 (28–197)	<.001
ALT (IU/L)			
1 month	123 (32–354)	80 (32–371)	.003
2 months	110 (53–306)	68 (35–262)	<.001
3 months	101 (12–242)	81 (20–110)	.003
GGT (IU/L)			
1 month	752 (161–2580)	750 (252–1782)	.55
2 months	973 (241–3320)	946 (192–1737)	.51
3 months	831 (241–2795)	812 (100–1580)	.53

Data were shown as medians(ranges).

The treatment of biliary atresia (BA) has improved in recent decades with early diagnosis, Kasai's operation, and appropriate postoperative management; however, approximately 60–80% of BA patients ultimately require liver transplantation [1–3]. As early reliable prediction of outcome will be helpful to plan a long-term strategy for BA treatment in each patient, this study aimed to determine an early, reliable predictor of the long-term outcome in order to optimize pretransplant management and to give parents a future prognostic estimation.

1. Patients and methods

Sixty-four cases of BA underwent Kasai's operation at our institution between 1990 and 2010. We excluded 10 cases from this study: 8 that died of another disease or anomaly and 2 with a native liver followed-up for less than 3 years. The medical charts of the remaining 54 cases of BA (23 boys) at our institute were reviewed with IRB approval. The median age at the time of operation was 55 days (7–120 days). Seven cases (13%) had type I atresia of the common bile duct and 47 (87%) had type III atresia at the porta hepatis, according to the BA classification schema of the Japanese Society of Pediatric Surgeons [4].

The 54 cases were divided into two groups based on their outcomes. In Group I, 30 cases required liver transplantation or died (n = 1), with a median age at liver transplantation or death of 1 year (4 months–19 years); in Group II, 24 cases lived with a native liver, with a median follow-up period of 9 years (5–20 years). Serum levels of total bilirubin (TB), direct bilirubin (DB), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyltransferase (GGT) measured sequentially before and 1, 2, and 3 months after Kasai's operation were compared between Groups I and II. Age at Kasai's operation and the macroscopic type of obstruction of the BA were also compared between the two groups. For cut-off determination, receiver operating characteristic (ROC) analysis was employed, and the area under the ROC curve (AUC), which is a common index of the reliability of the cut-off value derived from ROC analysis, was calculated for each biochemical parameter.

All data are presented as median values and ranges. The Mann–Whitney *U* test was used for comparison between Groups I and II. The Kruskal-Wallis H-test was used for comparison of the three groups defined after determination of cut-off values. Categorical variables were compared with the χ^2 test. Survival analysis of the BA cases with a native liver was carried out using the Kaplan-Meier method and log rank test. A significant difference was defined as $p < .05$.

2. Results

There was no significant difference between Group I and II in all preoperative data, including age at Kasai's operation and macroscopic type of obstruction, although the differences in DB ($p = .07$) and AST ($p = .06$) approached significance (Table 1). Serum levels of TB, DB, AST and ALT in Group I were significantly higher than those in Group II at 1, 2 and 3 months after operation ($p < .05$) (Table 2). There was no significant difference in GGT between the two groups at any time point. Therefore, four biochemical parameters (TB, DB, AST, ALT) were chosen for an ROC analysis of their relationship to the outcome at each time point. The AUC was widest at 2 months after operation compared with that at 1 and 3 months for all parameters (Table 3). The AUC of DB was highest compared with that of other parameters. According to the results, we determined DB at 2 months after operation to be the most

Table 3 AUC of biochemical data at each time point.

AUC	1 month	2 months	3 months
TB	0.83	0.92	0.88
DB	0.84	0.93	0.90
AST	0.81	0.83	0.80
ALT	0.68	0.78	0.69

reliable parameter. The cut-off value of DB determined by the ROC analysis was 0.7 mg/dl (sensitivity: 93%, specificity: 75%). The 5-, 10-, and 15-year survival rates of the BA cases with a native liver in cases with DB <0.7 mg/dl at 2 months after operation (n = 20; Group I:II = 2:18) were 90%, 90%, and 90%, respectively. The 5-, 10-, and 15-year survival rates in the BA cases with DB ≥0.7 mg/dl at 2 months after operation (n = 34; Group I:II = 28:6) were 41%, 30%, and 22%, respectively (log rank 17.9, p < .001) (Fig. 1). To clarify further details of BA outcomes, we added another parameter to the cut-off parameter: AST at 2 months after operation, for which the AUC was wider than that of ALT at every time point. The cut-off value of AST determined by the ROC analysis was 94 IU/L (sensitivity: 87%, specificity: 71%).

According to the cut-off values of DB and AST at 2 months after operation, the 54 cases were re-divided into three groups: group G (good) with DB <0.7 mg/dl and AST <94 IU/L (n = 16; Group I:II = 1:15); group M (moderate) with DB ≥0.7 mg/dl or AST ≥94 IU/L (n = 9; Group I: II = 4:5); and group P (poor) with DB ≥0.7 mg/dl and AST ≥94 IU/L (n = 29; Group I:II = 25:4). Age at operation, type of obstruction, and biochemical data at 2 months after operation of each group are shown in Table 4. Age at operation of group P was significantly higher than that of groups M and G (p = .04). The survival rates of the BA cases with a native liver in groups G, M, and P are shown in Fig. 2. In group G, one Group I case required liver transplantation at the age of one year, because of uncontrollable cholangitis causing acute severe liver dysfunction. In the remaining 15 Group II cases, 2 (13%) had an episode of cholangitis in the late follow-up period, and 13 did not encounter any complication related to BA. In group M, 5 cases had DB ≥0.7 mg/dl (group I:II = 3:2) and 4 cases had AST ≥94 IU/L (group I:II = 1:3) at 2 months after the operation. Four Group I cases included in group M underwent liver transplantation or died at a median age of 3.5 years (8 months - 13 years). In 5 Group II cases included in group M, 3 (60%) encountered complications, including cholangitis in 2 and pulmonary hypertension in 1. Twenty-five Group I cases included in group P underwent liver transplantation or died due to progressive liver dysfunction at a median age of 15 months (4 months–19 years). All 4 Group II cases included in group P suffered from various complications (esophageal varices in 2, cholangitis in 1, worsening liver function in 1). The 15-year survival rate in groups G, M, and P was 94%, 44%, and 22%, respectively (log rank 19.65, p < .001).

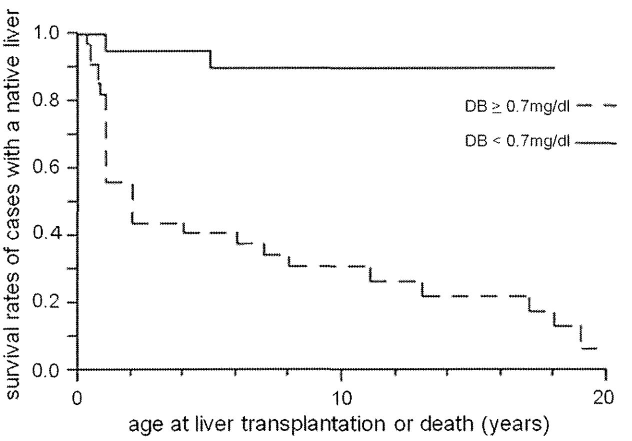

Fig. 1 Survival rate with native liver after Kasai's operation stratified by serum level of DB of 0.7 mg/dl at 2 months after operation.

3. Discussion

In this study, we tried to determine the most reliable prognostic factors and their timing of measurement after a Kasai's operation. The data we investigated in this study consisted of five biochemical parameters: serum TB and DB levels reflecting the degree of cholestasis, and AST, ALT,

Table 4 Preoperative data and biochemical parameters at 2 months after Kasai's operation.

	Group G	Group M	Group P	*P* value
age at operation (days)	47(7–80)	42 (29–71)	62(29–120)	.04
type of obstruction (I:III) *	3:13	1:8	3:26	.80
TB (mg/dl)	0.7 (0.3–1.2)	2.1 (0.5–8.8)	4.8 (1.7–15.5)	<.001
DB (mg/dl)	0.2 (0.1–0.6)	0.9 (0.2–5.9)	2.8 (0.7–11.2)	<.001
AST (IU/L)	68 (36–92)	88 (58–145)	132 (94–306)	<.001
ALT (IU/L)	62 (35–140)	74 (37–262)	108 (53–306)	<.001
GGT (IU/L)	958 (192–1737)	790 (234–2686)	978 (159–3320)	.75

Data were shown as medians(ranges).
* BA classification of the Japanese society of pediatric surgeons [4].

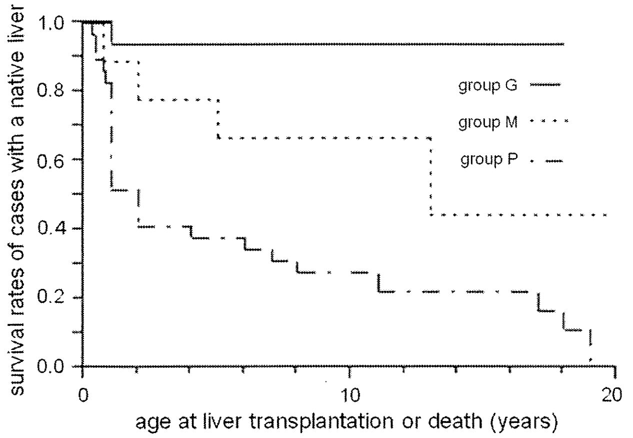

Fig. 2 Survival rate with native liver after Kasai's operation stratified by serum levels of DB of 0.7 mg/dl and AST of 94 IU/l at 2 months after operation.

and GGT levels reflecting inflammation and damage of liver cells and bile ducts. According to the results of the ROC analysis, DB and AST can be considered to be reliable predictive parameters for BA outcome.

Jaundice evaluated with serum TB level has been used as a predictive parameter of postoperative outcome after Kasai's operation [2,5–10]. Serum DB level has been rarely used as a parameter of BA outcome in previous publications, and was chosen based on the comparison of the AUC obtained from the ROC analysis in this study. Although TB has gained more popularity compared with DB, DB level reflects the degree of cholestasis more than does TB level, which is possibly influenced by indirect bilirubin level. The timing of evaluation of TB ranged between 6 weeks [6] and 6 months [2,9] in previous publications, including three reports that selected 3 months [5,7,8].

Uchida et al. reported the serum level of AST at 1 year after operation to be a predictive parameter for quality of life (QOL) and liver dysfunction in long-term jaundice-free survivors [11]. AST level is considered to be related to inflammation in liver cells, which possibly causes future liver fibrosis. The combination of DB and AST successfully revealed the BA outcome in detail, showing three clinical courses reported previously [5,11–13]: type 1, cases with progressive liver dysfunction finally requiring liver transplantation a few years after Kasai's operation; type 2, cases with no significant jaundice or liver dysfunction initially, but finally requiring liver transplantation owing to ongoing fibrosis; and type 3, cases that remain free from jaundice and liver dysfunction/portal hypertension which impair the QOL of BA patients. The clinical courses of these three types were considered to be comparable with groups P, M, and G separated by the cut-off values we determined in this study.

Previous publications have suggested other factors affecting the outcome of BA patients, including age at Kasai's operation [1,3,10,11,13–15], surgical decade [1–3], macroscopic type of BA [1,3,14], frequency of cholangitis [2,10,18], pathological findings of the liver at operation [12,16], caseload of the surgical center [17], presence or absence of intrahepatic cystic lesions [19], etc. Although many of these reports clarified the significance of each factor, their results remain controversial and uncertain, even the age at the operation which is one of the most commonly accepted factors as being strongly related to outcome. In this study, as in some other reports [2,11,12,16], we did not find significance of age at operation. Nio et al. suggested that the age at operation was significant for the short-term outcome of jaundice disappearance rate, but this difference might become smaller in the later period [15].

In conclusion, the combination of serum levels of DB and AST at 2 months after Kasai's operation is considered to be a reliable and useful predictor of the long-term outcome of BA. Prospective and large cohort studies are necessary to assess the usefulness of the predictive parameter we determined.

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Pediatr Surg Int (2013) 29:1159–1163
DOI 10.1007/s00383-013-3379-4

ORIGINAL ARTICLE

Impact of cow's milk allergy on enterocolitis associated with Hirschsprung's disease

Satoshi Umeda · Hisayoshi Kawahara · Akihiro Yoneda · Yuko Tazuke · Gakuto Tani · Tomohiro Ishii · Taro Goda · Katsuhisa Hirano · Kayo Ikeda · Shinobu Ida · Masahiro Nakayama · Akio Kubota · Masahiro Fukuzawa

Published online: 28 August 2013

Abstract
Purpose To investigate the impact of cow's milk allergy (CMA) on infants with Hirschsprung's disease (HD).
Methods Twenty-four patients, who developed gastrointestinal symptoms before the age of 60 days and underwent surgery for HD in the period between January 2003 and December 2012, were enrolled in this study. They were divided into two groups based on CMA-related findings: stimulation index of lymphocyte stimulation test >300 % and the presence of eosinophilic infiltration in the resected colon. Ten patients were determined specimen as not having CMA (Group A), because they did not satisfy any of the criteria. The remaining 14 were determined as having possible CMA (Group B), because they satisfied either or both findings. Patient background characteristics, pre- and postoperative clinical history, and laboratory data were compared between Groups A and B.
Results Pre- and postoperative enterocolitis did not occur in Group A patients. Postoperative enterocolitis was more frequent in Group B than in Group A ($p = 0.04$). Other clinical and laboratory data did not show significant difference between the two groups.
Conclusion CMA is a possible risk factor for postoperative enterocolitis in patients with HD.

Keywords Hirschsprung's disease · Cow's milk allergy · Lymphocyte stimulation test · Enterocolitis

S. Umeda (✉) · H. Kawahara · A. Yoneda · Y. Tazuke · G. Tani · T. Ishii · T. Goda · K. Hirano · A. Kubota · M. Fukuzawa
Department of Pediatric Surgery, Osaka Medical Center and Research Institute for Maternal and Child Health, 840 Murodo-cho Izumi, Osaka 594-1101, Japan
e-mail: s_umeda3104@yahoo.co.jp

K. Ikeda · S. Ida
Department of Pediatric Gastroenterology and Nutrition, Osaka Medical Center and Research Institute for Maternal and Child Health, 840 Murodo-cho Izumi, Osaka 594-1101, Japan

M. Nakayama
Department of Pathology, Osaka Medical Center and Research Institute for Maternal and Child Health, 840 Murodo-cho Izumi, Osaka 594-1101, Japan

Introduction

Recently, the clinical entities of gastrointestinal food allergy such as the food protein-induced enterocolitis syndrome (FPIES) have become well known. FPIES is an uncommon pediatric, non-immunoglobulin E (IgE)-mediated disorder triggered by the ingestion of certain food proteins and is thought to be related with food protein stimulation of T cells in the gastrointestinal mucosa [1]. Cow's milk is one of the most popular food antigens possibly causing FPIES in small infants [2].

We have previously reported an infant, with the misdiagnosis of Hirschsprung's disease (HD), in whom normal ganglia were found in the resected rectum and cow's milk allergy (CMA) developed after undergoing Soave procedure [3]. This experience taught us the importance of examination for CMA in patients with HD-like gastrointestinal symptoms. On the other hand, some patients are first diagnosed with CMA and HD is found subsequently. In this study, we retrospectively investigated the impact of CMA on the clinical condition of HD patients.

Materials and methods

With IRB approval, the medical charts of 24 patients (18 boys) who presented with gastrointestinal symptoms before the age of 60 days and underwent surgery for HD in our institute in the period between January 2003 and December 2012 were reviewed. Their median age at the time of symptom onset was 5 days, ranging from 0–56 days. The location of the aganglionic segment was rectosigmoid ($n = 19$, 79 %), descending colon ($n = 3$, 12 %), transverse colon ($n = 1$, 4 %), and total colon ($n = 1$, 4 %). All patients were nourished with pediatric elemental diet (Elental P ™) after the diagnosis of HD was made. Their median age at the time of operation was 69.5 days, ranging from 19 to 793 days. Two patients underwent open Soave procedure, 7 required laparoscopic assist, and the remaining 15 underwent transanal Soave procedure (including one patient who finally underwent transanal Swenson procedure).

The 24 patients were divided into two groups based on CMA-related laboratory findings: the value of lymphocyte stimulation test (LST) and eosinophilic infiltration in the colon obtained at surgery for HD or rectal suction biopsy.

LST measures the incorporation of tritiated thymidine during DNA synthesis in the proliferation of T cells associated with reactions to drugs or foods, and is aimed at evaluating non-IgE-mediated allergy. Our previous report indicated that LST was interpreted as positive when the stimulation index (SI) of LST was greater than 300 % [4, 5]. Eosinophilic infiltration was defined by histopathological findings showing a minimum of 15 eosinophils per high-power field in the epithelium.

The median value of SI of LST was 479 %, ranging from 100 to 2,069 %. Ten patients showed SI of LST higher than 300 %, and these results were interpreted as positive. Eosinophilic infiltration was present in the colon obtained at surgery for HD or rectal suction biopsy specimen in eight patients. Four patients showed positive results for both LST and histopathological examination. Six patients showed a positive result only in LST, and four patients showed a positive result only in histopathological examination. So, 10 patients were determined not to have CMA based on the above criteria (Group A) and the remaining 14 were determined to have possible CMA (Group B).

Patient background characteristics, preoperative and postoperative clinical history, laboratory data, including white blood cell (WBC), eosinophil (Eo), C-reactive protein (CRP), and LST, and plain abdominal X-ray findings were compared between Groups A and B. In the plain abdominal X-ray, intestinal dilation was defined as positive when the largest diameter of the intestine was wider than half the diameter of the lumbar vertebrae. The presence of enterocolitis was determined as positive when a patient suffered from enterocolitis requiring in-hospital treatment.

Data are shown as median value and range. Statistical analyses were conducted with Mann–Whitney *U*-test and Fischer's exact probability test. A value of $p < 0.05$ was considered significant.

Results

Patient background characteristics

Male patients were more frequent in both groups; 6 of 10 in Group A and 12 of 14 in Group B, but the difference between the two groups was not significant ($p = 0.19$).

Rectosigmoid colon aganglionosis was prevalent in both groups; 9 (90 %) in Group A and 10 (71 %) in group B. Descending colon aganglionosis was present in one patient in Group A and two in Group B. The remaining two patients in Group B had transverse colon aganglionosis and total colon aganglionosis, respectively. The prevalence of the different types of aganglionosis was not significantly different between the two groups ($p = 0.67$).

Two patients had other congenital anomalies in Group A: one had a chromosomal anomaly (trisomy 22) and the other had Mowat–Wilson syndrome. On the other hand, three patients had other congenital anomalies in Group B: one had congenital heart disease (asplenia syndrome and univentricular heart) and a gastrointestinal anomaly (malrotation), one had a chromosomal anomaly (trisomy 21) and gastrointestinal anomalies (duodenal atresia and malfusion of pancreaticobiliary ducts), and another had a chromosomal anomaly (trisomy 21). The prevalence of other congenital anomalies was not significantly different between Groups A and B ($p = 1.0$).

Preoperative clinical conditions and laboratory data (Table 1)

There was no significant difference in all preoperative conditions except SI for cow's milk by LST between Groups A and B ($p = 0.01$). Intestinal dilatation on X-ray was more frequent in Group B than in Group A, but the difference did not reach statistical significance ($p = 0.21$) (Fig. 1). Preoperative enterocolitis developed in no patient in Group A and 4 (29 %) in Group B, the incidence of which did not show a significant difference ($p = 0.11$). There was no significant difference in LST ($p = 0.42$) and the histology ($p = 0.73$) between four patients with enterocolitis and ten patients without enterocolitis in Group B.

Table 1 Preoperative clinical condition and laboratory data

	Group A ($n = 10$)	Group B ($n = 14$)	p value
Age at appearance (days)	2 (0–56)	10.5 (0–46)	0.10
Complaints at appearance			
Non-bile vomiting	6 (60 %)	6 (43 %)	0.68
Bile vomiting	1 (10 %)	4 (29 %)	0.36
Abdominal distention	8 (80 %)	6 (43 %)	0.10
Poor oral intake	1 (10 %)	6 (43 %)	0.17
Complaints on the admission			
Vomiting (not bile like)	1 (10 %)	3 (21 %)	0.61
Vomiting (bile like)	5 (50 %)	6 (43 %)	1.0
Abdominal distention	10 (100 %)	14 (100 %)	1.0
Blood test on the first visit			
White blood cell (/μl)	11,700 (6,000–22,600)	12,600 (5,200–18,100)	0.60
Eosinophil (/μl)	150 (0–1,064) ($n = 8$)	252 (86–1,724) ($n = 10$)	0.29
Eosinophil (%)	1 (0–7) ($n = 8$)	3 (1–12) ($n = 10$)	0.19
C-reactive protein (mg/dl)	0.2 (0.05–14.6) ($n = 9$)	0.1 (0.03–1.06) ($n = 9$)	0.11
SI for cow's milk by LST	152 (100–236) ($n = 3$)	583 (397–2,069) ($n = 10$)	0.01
% Body weight before surgery (%)	101 (79–112) ($n = 8$)	101.6 (60.9–117.7) ($n = 14$)	0.73
Enterocolitis	0 (0 %)	4 (29 %)	0.11
Number of times of enterocolitis, median (range)	0	0 (0–1)	0.07
Preoperative period	63 (23–171)	50 (6–773)	0.73
Pediatric ED feeding (days)	56 (19–115)	36 (6–148)	0.47
Intestinal dilatation on X-ray	3/10 (30 %)	9/14 (64 %)	0.21
Age of rectal biopsy (days)	6 (3–59)	18 (5–719)	0.07
Age of operation (days)	66 (25–171)	77 (19–793)	0.52

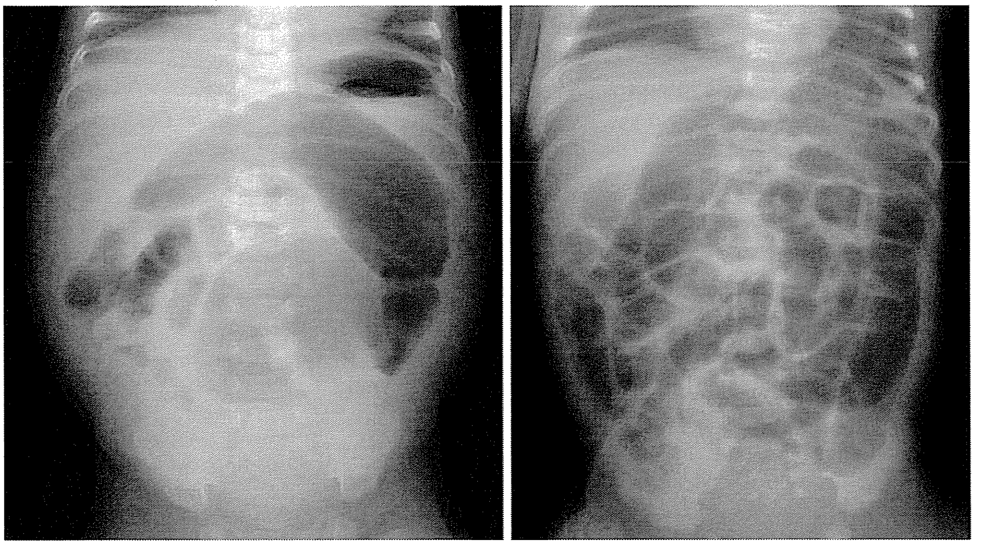

Fig. 1 Plain abdominal X-ray of a Group A patient, showing a dilated intestinal gas shadow mainly in the colon (*left*), and that of a Group B patient, showing a dilated intestinal gas shadow in both the small intestine and colon (*right*)

Postoperative clinical conditions and laboratory data (Table 2)

Enterocolitis did not occur in Group A patients, but occurred in 5 (36 %) Group B patients ($p = 0.05$). Enterocolitis was more frequent in Group B than in Group A ($p = 0.04$). The period of elemental formula feeding was significantly longer in Group B than in Group A ($p = 0.02$). There was no significant difference in medication at 6 months after operation between Groups A and B.

Table 2 Postoperative clinical condition and laboratory data

	Group A	Group B	*p* value
Enterocolitis	0 (0 %)	5 (36 %)	0.05
Number of times of enterocolitis, median (range)	0	0 (0–1)	0.04
Period of elemental formula feeding (days)	$n = 9$ 118 (8–1,287)	$n = 14$ 156 (10–1,868)	0.02
Medication at 6 months after operation	$n = 9$	$n = 14$	
No medication	3 (33 %)	2 (14 %)	0.34
Glycerin enema	4 (44 %)	7 (50 %)	1.0
Laxative	5 (56 %)	9 (64 %)	1.0

Discussion

CMA patients often present with various gastrointestinal symptoms, and it is sometimes difficult to discriminate CMA from HD [5]. The incidence of CMA is reported to be 0.21 to 3 % [6–9]. It was reported that the incidence of CMA in infants with birth weight <1,000 g was significantly higher than that in infants with birth weight of 1,500–2,500 g [8]. The diagnosis of CMA was correctly made if gastrointestinal symptoms were relieved by cow's milk elimination and appeared on challenge test. Because non-IgE-mediated delayed type allergic reactions are thought to play a predominant role in the majority of CMA, cow's milk-specific IgE antibody level has a limited role as an indicator of CMA. Therefore, LST has been proposed as an alternative diagnostic test for CMA [10, 11]. Ikeda et al. [4] proposed that LST is interpreted as positive when SI is greater than 300 %, based on the receiver operating characteristic curve obtained from the data of LST values of CM in 94 infants with and without CMA (sensitivity 95 %, specificity 69 %). Most patients classified as having possible CMA in this study showed SI of LST much higher than 300 %.

Comparing clinical and laboratory data between non-CMA and possible CMA patients, the frequency of postoperative enterocolitis and the period of elemental formula feeding were significantly greater in possible CMA patients. Allergic characteristics, e.g. a positive result of LST, presence of eosinophilic infiltration in the colon resected at surgery or rectal suction biopsy specimen, are considered to be risk factors for enterocolitis in HD patients. Our finding that non-CMA patients did not have a history of enterocolitis before or after the Soave procedure needs to be validated in a larger number of HD patients without CMA.

The incidence of preoperative enterocolitis in HD patients was reported to be 15 to 50 %, and postoperative enterocolitis occurs in 2 to 33 % of patients [12]. Many risk factors, including increased length of the aganglionic segment, female sex, trisomy 21, and the presence of other associated congenital anomalies, have been identified and help support the current model for the pathophysiology of enterocolitis associated with HD [13–16], whereas, to our knowledge, CMA has not been proposed as a risk factor for enterocolitis in HD patients.

It was not clarified in this study whether the HD patients had allergy for food antigens other than cow's milk, because LST for other foods was not conducted in the HD patients. Some patients were examined with radioallergosorbent test (RAST) for IgE-mediated food allergy. One patient of Group A showed a positive result of RAST for egg white at 99 days of age. Six patients of Group B showed positive results of RAST for egg white, and two of the six showed positive results of RAST for egg yolk at the median age of 511 days (234–1,385 days). The correct incidence of co-existing other food allergy remains unknown, because systematic follow-up study was not conducted in the patients.

In conclusion, examination of the association of CMA is worthwhile, and it is a possible risk factor for enterocolitis in HD patients. Large, prospective cohort studies are needed to clarify the clinical impact of CMA on HD patients.

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Peptides 43 (2013) 160–166

Contents lists available at SciVerse ScienceDirect

Peptides

journal homepage: www.elsevier.com/locate/peptides

Ghrelin and glucagon-like peptide-2 increase immediately following massive small bowel resection

Mitsuru Muto[a], Tatsuru Kaji[a,*], Motoi Mukai[a], Kazuhiko Nakame[a], Takako Yoshioka[b], Akihide Tanimoto[b], Hiroshi Matsufuji[a]

[a] Department of Pediatric Surgery, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima 8908520, Japan
[b] Department of Molecular and Cellular Pathology, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima 8908520, Japan

ARTICLE INFO

Article history:
Received 21 December 2012
Received in revised form 1 March 2013
Accepted 1 March 2013
Available online 18 March 2013

Keywords:
Acyl ghrelin
Des-acyl ghrelin
GLP-2
Adaptation
Massive small bowel resection
Short bowel syndrome

ABSTRACT

Children with short bowel syndrome face life-threatening complications. Therefore, there is an urgent need for a new therapy to induce effective adaptation of the remnant intestine. Adaptation occurs only during feeding. We focused on preprandial acyl ghrelin and des-acyl ghrelin, and postprandial glucagon-like peptide-2 (GLP-2), which are known to have active orexigenic and trophic actions. This study aims to clarify the secretion trends of these hormones after massive small bowel resection and to obtain basic data for developing a new treatment. Sixty-three growing male rats were used: 3 were designated as controls receiving no operation and 60 were randomized into the 80% small bowel resection (80% SBR) group and the transection and re-anastomosis group. Changes in body weight, food intake, and remnant intestine morphology were also assessed for 15 days after the operation. Acyl ghrelin and des-acyl ghrelin levels increased immediately, equivalently in both operation groups ($P = 0.09$ and 0.70). Interestingly, in 80% SBR animals, des-acyl ghrelin peaked on day 1 and acyl ghrelin peaked on day 4 ($P = 0.0007$ and $P = 0.049$ vs controls). GLP-2 secretion was obvious in 80% SBR animals ($P = 2.25 \times 10^{-6}$), which increased immediately and peaked on day 4 ($P = 0.009$ vs. controls). Body weight and food intake in 80% SBR animals recovered to preoperative levels on day 4. Morphological adaptations were evident after day 4. Our results may suggest a management strategy to reinforce these physiological hormone secretion patterns in developing a new therapy for short bowel syndrome.

1. Introduction

The loss of a large part of the small bowel in infants, owing to surgical removal or a congenital defect, leads to a condition called short bowel syndrome. The 3 most common causes of short bowel syndrome in children are necrotizing enterocolitis, intestinal atresia, and midgut volvulus [22]. When a large part of the small intestine is lost, the functional ability of the remaining intestine is often inadequate to support growth and hydration, and prolonged parenteral nutritional support is required. Children with a short bowel are at risk for many life-threatening complications such as sepsis due to catheter-related blood stream infection and parenteral nutrition-associated liver disease even when these children are under total parenteral nutrition. In the clinic, decisions about the optimal management of pediatric short bowel syndrome are often based on repeated trial-and-error treatments, depending on the condition of a specific patient. Therefore, there is an urgent need for a new therapy to compensate for the lost functionality of the small intestine.

Fundamentally, when a large section of the small intestine is lost, the reduction in nutritional absorption is compensated gradually by an increase in the mucosal surface area of the remaining bowel, accompanied by increases in the villus height and crypt cell proliferation rates. This process is known as adaptation [32]. The regulation and augmentation of the function of the remaining intestine is induced through a complex interaction of many different factors, including luminal nutrients and gastrointestinal hormones [22,28]. Physiologically, bowel adaptation is supposed to occur only in response to oral feeding [32]. In this study, we investigated the levels of 3 gastrointestinal hormones, acyl ghrelin, des-acyl ghrelin, and glucagon-like peptide-2 (GLP-2).

Ghrelin is secreted by the X/A-like cells of the stomach and the proximal small intestine. Two major molecular forms of ghrelin exist, of which acyl ghrelin with n-octanoylated modification appears to serve multiple functions [7,15,27], including exerting positive effects on food intake, growth hormone

Abbreviations: GLP-2, glucagon-like peptide-2; 80% SBR, 80% small bowel resection.

* Corresponding author at: Department of Pediatric Surgery, Kagoshima University Graduate School of Medical and Dental Sciences, 8-35-1 Sakuragaoka, Kagoshima 8908520, Japan. Tel.: +81 99 275 5444; fax: +81 99 275 2628.
E-mail address: tatu@m2.kufm.kagoshima-u.ac.jp (T. Kaji).

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http://dx.doi.org/10.1016/j.peptides.2013.03.006

secretory action, glucose and lipid metabolism, gastrointestinal motility, cell proliferation, and hemodynamics, all of which may contribute to intestinal adaptation after massive small bowel resection. On the other hand, non-acylated des-acyl ghrelin induces a negative energy balance by decreasing food intake and delaying gastric emptying [2]. Furthermore, des-acyl ghrelin suppresses acyl ghrelin-induced food intake [10]; a continuous infusion of des-acyl ghrelin is reported to reduce weight gain [21].

GLP-2 is secreted by the intestinal L-cells of the distal ileum and proximal colon in response to both direct stimulation of luminal nutrients and vagally mediated pathways, which are activated by the presence of nutrients in the proximal bowel [12]. GLP-2 is best known for its beneficial role in intestinal adaptation and has become a focus of studies on short bowel syndrome [32]. A randomized placebo-controlled study of teduglutide, a GLP-2 analog, showed a potential reduction in the dependency on parenteral support of adult patients with short bowel syndrome [11]. However, this treatment has not been applied clinically in children.

The purpose of this study was to clarify the trends in the secretion of endogenous acyl ghrelin, des-acyl ghrelin, and GLP-2 following massive small bowel resection in order to obtain basic data for the future investigation of a new treatment that may induce efficient intestinal adaptation in patients with short bowel syndrome.

2. Materials and methods

2.1. Animals

Sixty-three 7-week-old male Sprague-Dawley rats weighing 200–240 g (purchased from Kyudo Co., Ltd., Saga, Japan) were used in this experiment. The animals were individually housed in cages with free access to standard rat chow and water, and maintained under standardized temperature (23 °C ± 1 °C), humidity (50% ± 10%), and 12-h light-dark cycles (lights on at 7:00 a.m.).

All experimental procedures were approved by the Laboratory Animal Committees of Kagoshima University Graduate School and were performed in accordance with the "Guidelines for the Care and Use of Laboratory Animals."

2.2. Study design

Sixty animals were randomized to either the 80% small bowel resection (SBR) group or the transection and re-anastomosis operation (sham) group and allowed to acclimatize to their environment for 6 days before experimentation. Changes in body weight, food intake, water intake, amount of stool, and amount of urine were measured from 7:00 to 8:00 a.m. throughout the experimental period. Preprandial plasma acyl ghrelin and des-acyl ghrelin levels, postprandial plasma GLP-2 levels, and intestinal morphology were assessed at days 1, 4, 7, 11, and 15 after the operation (6 animals per day for the 2 operation groups). As a control, the same measurements described above were assessed in 3 animals at day 0. Plasma acyl and des-acyl ghrelin levels are known to fluctuate according to psychological or physical stresses [16,34]; therefore, the environments of the experimental animals were noted to be uniform. The 80% SBR animal model has been well established [18,37]. Adaptive response in an 80% SBR rat model was reported to be most pronounced in the first week [18], and the morphological changes reached a plateau (equivalent to 30-postoperative-day levels) within 12 days in a 70% SBR rat model [9]. Thus, we set 15 days as the experimental period for this study. The promotion of functional alterations in response to morphological adaptations following massive small bowel resection has been previously reported [19,26,37].

2.3. Surgical methods

The animals were fasted overnight, anesthetized with isoflurane (1.5% inhalation by mask), and explored through a midline laparotomy under sterile conditions. Intestinal length was measured in a standardized fashion, and 80% SBR was performed, leaving 15 cm of the ileum above the ileocecal valve anastomosed to the jejunum 5 cm below the ligament of Treitz [13]. Bowel anastomoses were completed with the aid of an operating microscope, using interrupted 6-0 silk sutures (Alfresa Pharma Corporation, Tokyo, Japan), and the abdominal incision was closed with 3-0 polyglycolic sutures (Johnson & Johnson K.K., Tokyo, Japan). Sham-operated rats were transected at 15 cm above the ileocecal valve and re-anastomosed [13].

All animals received cefazolin (50 mg/kg per dose subcutaneously; Otsuka Pharmaceutical Factory, Inc., Tokushima, Japan) to prevent postoperative infection and buprenorphine (0.01 mg/kg per dose subcutaneously; Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) for analgesia [13]. Additionally, a subcutaneous injection of 10 mL isotonic saline was given to prevent postoperative dehydration. The animals were allowed free access to water immediately after surgery and standard rat chow ad libitum at the dark cycle of the first postoperative day.

2.4. Measurement of plasma acyl ghrelin, des-acyl ghrelin, and GLP-2 levels

After an overnight fast, rats were anesthetized by isoflurane inhalation. Blood was obtained from the tail between 10:00 and 12:00 a.m. then immediately centrifuged at 1500 × g for 15 min at 4 °C. All plasma samples were stored at −80 °C until assayed.

2.4.1. Preprandial acyl ghrelin and des-acyl ghrelin

For the acyl ghrelin and des-acyl ghrelin assay, blood samples were drawn into chilled polypropylene tubes containing 0.2 M ethylenediaminetetraacetic acid, disodium salt (EDTA·2Na) (20 μL/1 mL blood sample) and aprotinin (0.3–0.8 trypsin inhibitor unit/1 mL of blood sample) and then centrifuged. Aliquots of plasma were acidified with 1 N hydrogen chloride and then stored. Plasma acyl ghrelin and des-acyl ghrelin levels were measured using an enzyme-linked immunosorbent assay kit (Mitsubishi Chemical Medicine Corporation, Tokyo, Japan).

2.4.2. Postprandial GLP-2

One hour after gavage of the animals with 2 mL of a liquid meal (ENSURE H; Abbott Japan Co., Ltd., Tokyo, Japan), blood samples were drawn into chilled polypropylene tubes containing 0.2 M EDTA·2Na (20 μL/1 mL blood sample) and centrifuged. Aliquots of plasma were stored. Plasma GLP-2 levels were measured by an enzyme immunoassay kit (Yanaihara Institute Inc., Shizuoka, Japan).

2.5. Gross intestinal morphology and histology

After the collection of blood for the GLP-2 assay, the animals were euthanized by exsanguination. The total small intestine was harvested for gross and microscopic morphological analysis.

The mesentery was removed, and the total length of the small bowel was measured from the ligament of Treitz to the ileocecal valve along the antimesenteric border. The harvested small intestine was quickly opened along the mesenteric border, rinsed in cold saline, and then weighed. Bowel width was measured at the middle point of the opened jejunum and ileum. Samples for microscopic analysis were harvested from the jejunum (2.5 cm below the ligament of Treitz), the proximal ileum (12.5 cm above the ileocecal valve, i.e., 2.5 cm below the anastomotic line), and

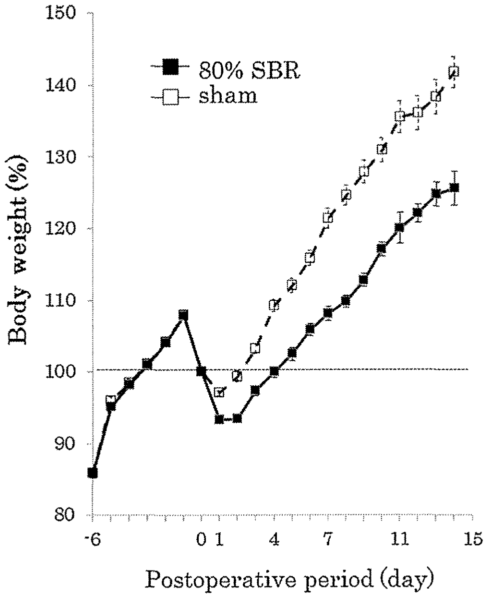

Fig. 1. Changes in body weight. Body weight was set at 100% at the time of the operation. Data are reported as the means ± standard error. A significant difference ($P < 0.01$) in body weight was found between the 80% SBR group and the sham operation group throughout the postoperative period, as analyzed by Student's *t*-test. 80% SBR, 80% small bowel resection.

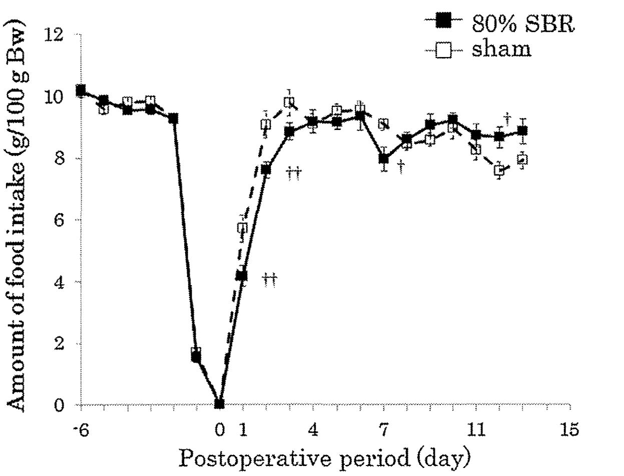

Fig. 2. Changes in the amount of food intake. Data are shown as the mean value of intake per 100 g of body weight ± standard error. The statistical differences between the groups were analyzed by Student's *t*-test. ††$P < 0.01$ and †$P < 0.05$ versus the sham operation group at similar time points. 80% SBR, 80% small bowel resection.

the distal ileum (2.5 cm above the ileocecal valve) and fixed in a 10% formaldehyde neutral buffer solution for 24 h. Mucosal scrapings from the residual jejunum and ileum were weighed. Paraffin sections of formalin-fixed tissue were cut at 3-μm thickness and stained with hematoxylin and eosin. For each sample slide, microscopic measurements of the villus height, villus width, crypt depth, and number of villi per 1 mm were recorded from 5 well-oriented villi/crypt units. The quantification was performed with the help of an expert pathologist. The absorptive mucosal surface area per 1 cm^2 of the intestine was calculated using methods discussed previously. In brief, the mucosal surface area was calculated by first considering the intestine as a cylinder and then multiplying the added mucosal surface area contributed by the villi, considering each villus as a cone [19,23].

2.6. Statistical analysis

Data are presented as the mean values ± standard error (SE). Statistical analyse between groups and time courses were performed by 2-factor factorial analysis of variance (ANOVA) followed by Tukey's multiple-comparison posttest. Comparisons with controls were performed by Dunnett's test. Comparisons between the experimental groups at similar time points were performed by Student's *t*-test. Statistical analysis was completed using Ekuseru-Toukei 2010 (Social Survey Research Information Co., Ltd., Tokyo). All results were considered statistically significant when *P* values were <0.05.

3. Results

3.1. Changes in daily assessment data

The body weight of animals in the 80% SBR group returned to preoperative levels within 4 days, and continued to increase steadily (Fig. 1). Food intake in the 80% SBR animals recovered to preoperative levels on postoperative day 4. After day 4, roughly equivalent intake was maintained between the 2 operative groups, the 80% SBR animals and the sham-operated animals (Fig. 2). The sham-operated animals showed higher water intake, amount of stool, and amount of urine than the 80% SBR animals until postoperative day 4. Subsequently, these measures remained almost the same in both groups (data not shown). Loose stools were observed in the 80% SBR animals several days after the operation; however, watery or muddy stools were not observed.

3.2. Changes in intestinal morphology

Gross morphological changes in bowel weight and mucosal weight were evident in the 80% SBR animals after postoperative day 4 compared with controls. Moreover, these changes were significantly different in comparisons with the sham-operated animals at similar time points (Table 1). The increase in villus height of the 80% SBR animals was more evident than that of the sham-operated animals after postoperative day 4 (Fig. 3). In the microscopic quantification, villus height and crypt depth were significantly increased after day 4 in the 80% SBR animals (Table 2). There were no significant differences in the changes of villus width or the number of villi per 1 mm (data not shown). The increase in absorptive mucosal surface per unit area was based on the growth of the villus height. The expansion of absorptive mucosal surface was observed starting on day 4 (Table 2).

3.3. Changes in gastrointestinal hormone levels

The levels of all 3 gastrointestinal hormones immediately increased following massive small bowel resection compared with the controls.

In 80% SBR animals, preprandial plasma acyl ghrelin peaked on day 4, with a significant difference versus controls (means ± standard error, 104.7 ± 14.1 fmol/mL, $P = 0.049$). The time when acyl ghrelin reached its peak level accorded with the time when the body weight and food intake recovered to the preoperative levels. It also matched the timing when the morphology of the remaining intestine began to change significantly. Interestingly, the peak of preprandial plasma des-acyl ghrelin was observed on day 1 (1021.6 ± 93.1 fmol/mL, $P = 0.0007$ vs. control). Concerning preprandial acyl ghrelin and des-acyl ghrelin, equivalent plasma levels were maintained in 80% SBR animals, i.e., under short bowel conditions, and in sham-operated animals, i.e., under native small bowel length conditions ($P = 0.09$ and $P = 0.70$).

The postprandial plasma GLP-2 concentration in 80% SBR animals peaked on day 4, with a significant difference versus controls

Table 1
Changes in gross intestinal morphology.

			Jejunum				Ileum			
		n	Bowel length (cm)	Bowel width (cm)	Bowel weight (mg/cm)	Mucosal weight (mg/cm)	Bowel length (cm)	Bowel width (cm)	Bowel weight (mg/cm)	Mucosal weight (mg/cm)
Control		3	5.0 ± 0.0	1.0 ± 0.0	101.3 ± 6.4	38.5 ± 4.3	15.0 ± 0.0	1.0 ± 0.0	105.6 ± 7.2	44.5 ± 4.1
80%SBR	Day 1	6	5.1 ± 0.1	1.1 ± 0.1	95.8 ± 2.3	41.5 ± 4.0	15.2 ± 0.4	1.2 ± 0.1	104.9 ± 6.3	43.7 ± 2.7 †
	Day 4	6	5.2 ± 0.2	1.2 ± 0.1	142.9 ± 10.9 ††	60.1 ± 6.0 ††	15.8 ± 0.5	1.4 ± 0.0 **,††	131.5 ± 2.7 ††	57.5 ± 2.7 ††
	Day 7	6	5.2 ± 0.1	1.4 ± 0.1 **,††	166.0 ± 13.8 *,††	58.4 ± 4.2 ††	16.6 ± 0.8	1.5 ± 0.1 **,††	177.5 ± 9.4 **,††	73.7 ± 4.0 **,††
	Day 11	6	5.1 ± 0.2	1.6 ± 0.0 **,††	261.5 ± 25.4 **,††	98.9 ± 12.1 **,††	16.4 ± 0.2	1.8 ± 0.0 **,††	208.0 ± 10.8 **,††	78.9 ± 4.8 **,††
	Day 15	6	5.3 ± 0.3	1.7 ± 0.1 **,††	267.3 ± 21.2 **,††	93.2 ± 11.3 **,††	15.7 ± 0.3	1.7 ± 0.0 **,††	223.9 ± 8.9 **,††	89.2 ± 4.8 **,††
Sham	Day 1	6	5.0 ± 0.0	1.0 ± 0.0	86.0 ± 10.1	30.4 ± 5.2	14.9 ± 0.1	1.1 ± 0.0	90.3 ± 3.1	34.2 ± 2.5
	Day 4	6	5.0 ± 0.0	1.1 ± 0.0	87.0 ± 3.4	32.5 ± 2.0	15.3 ± 0.5	1.1 ± 0.0	90.5 ± 3.6	36.4 ± 3.2
	Day 7	6	5.0 ± 0.0	1.0 ± 0.0	93.4 ± 3.6	34.9 ± 1.8	16.3 ± 0.5	1.1 ± 0.0	110.2 ± 7.5	40.5 ± 4.3
	Day 11	6	5.0 ± 0.0	1.0 ± 0.0	100.3 ± 3.7	37.4 ± 1.8	15.7 ± 0.2	1.1 ± 0.0	133.7 ± 7.8	46.3 ± 3.4
	Day 15	6	5.0 ± 0.0	1.1 ± 0.1	106.3 ± 5.0	40.8 ± 3.2	15.7 ± 0.4	1.2 ± 0.1	133.4 ± 8.2	43.2 ± 2.6

Data are expressed as the means ± SE. Comparisons with controls were performed by Dunnett's test. Comparisons among the groups at similar time points were analyzed by Student's *t*-test.
* $P < 0.05$ versus controls.
** $P < 0.01$ versus controls.
† $P < 0.05$ versus the sham operation subjects.
†† $P < 0.01$ versus the sham operation subjects.
80% SBR, 80% small bowel resection.

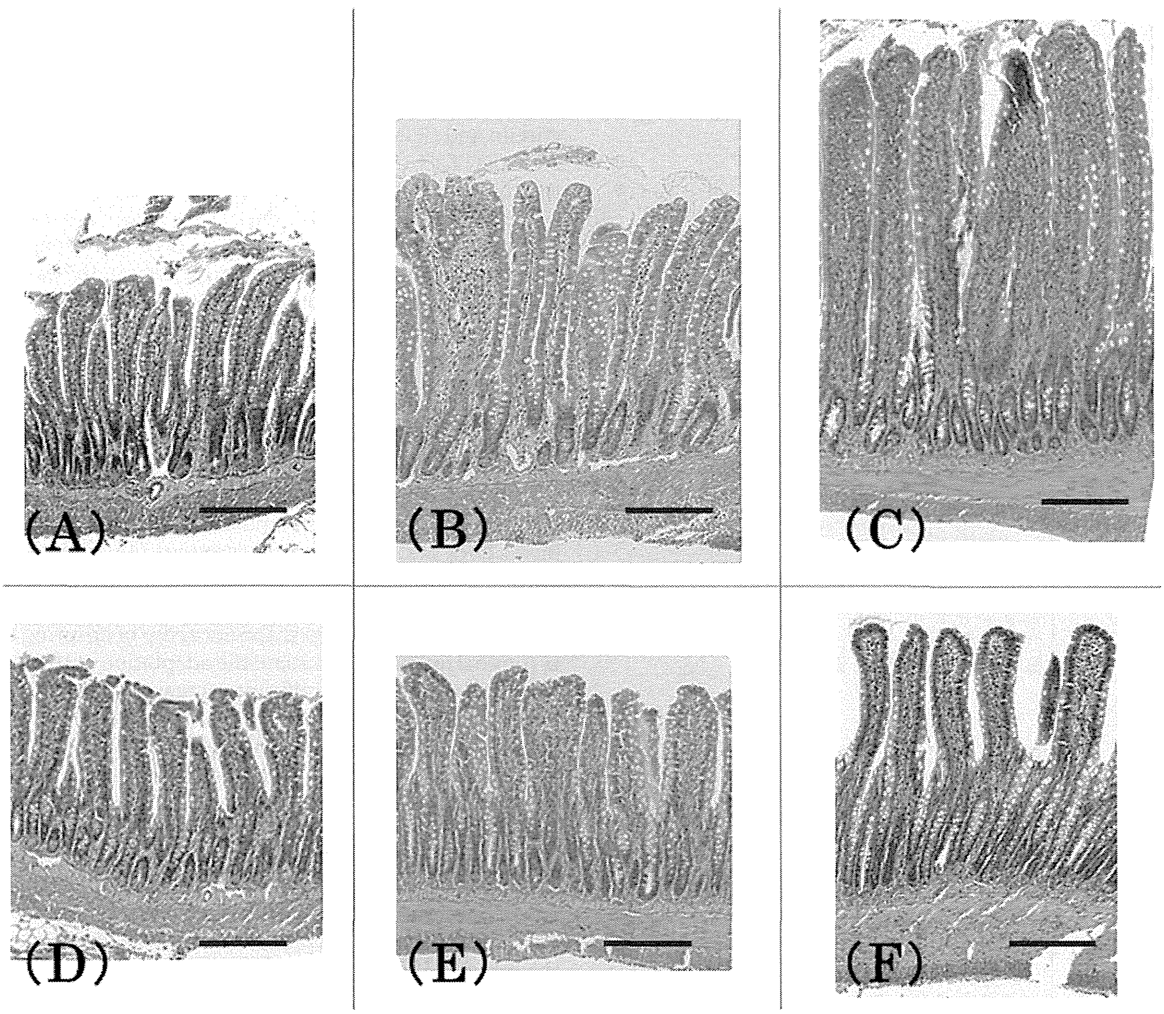

Fig. 3. Changes in intestinal morphology in the proximal ileum. Morphological changes were evident after day 4 in the 80% SBR animals but not in sham-operated animals. This tendency was seen in both the remaining jejunum and ileum. The changes in microscopic morphology in the proximal ileum (12.5 cm above the ileocecal valve) are shown. (A) Post 80% SBR on day 1; (B) post 80% SBR on day 4; (C) post 80% SBR on day 15; (D) post sham operation on day 1; (E) post sham operation on day 4; (F) post sham operation on day 15. Bar, 200 μm. 80% SBR, 80% small bowel resection.