置は、20〜40秒程度で設定圧力値まで達し、一定時間の高圧処理後、数秒で大気圧まで減圧する事ができものである（図1）。非加圧（大気圧）、および300MPaで加圧処理した新鮮凍結血漿は、加圧処理後すぐに凍結し、凝固因子活性測定直前に融解して試験に用いた。ウイルス液は加圧処理後すぐに試験に用いた。

3.凝固、抗凝固因子の活性測定

活性化部分トロンボプラスチン時間(APTT),プロトロンビン時間(PT),第8因子活性、凝固第9因子活性、フィブリノゲン濃度は、それぞれ、データファイ・APTT、ネオプラスチン・プラス、第8因子欠乏血漿、第9因子欠乏血漿、データファイ・フィブリノーゲンを用い、血液凝固測定装置で測定した。アンチトロンビンIIIは測定キットを用いて生化学的自動分析装置で測定した。第7因子、第11因子、第13因子、フォンビルブラント因子、プラスミノーゲン、プロテインC、プロテインSの活性測定は、株式会社エスアールエルに依頼した。

4.不活化評価

(1) ヒトパルボウイルスB19

B19ウイルスとしてB19陽性血漿を用いた。2x 10^5個のNEC細胞(ヒト胎児性ガン細胞)を感染1日前に24穴プレートに撒き、10%FCSを含んだRPMIで段階希釈した加圧処理ウイルス溶液、および非加圧ウイルス溶液を細胞に添加した。ウイルスの吸着効率を高めるためにポリブレンを最終濃度5μg/mLになるように加え、37℃に設定したCO_2インキュベーター内で培養しウイルスを細胞へ吸着させた。2時間後、1mLの10%FCS-RPMI培養液を加え2日間培養し、細胞からRNAを抽出した。RNAは15μLに溶解し、5μLを用いてnested RT-PCRを行い、感染することで生じるspliced RNAが検出された最大希釈倍率の逆数を感染価とした。

(2) 日本脳炎ウイルスJEV

JEVとしてはBeijing-1 smb37株を用いた。6x 10^5個のVero9013細胞を感染1日前に6穴プレートに撒き、2%FBSを含んだEMEM培地で段階希釈した加圧処理ウイルス溶液、及び非加圧ウイルス溶液を細胞に添加した。37℃で1時間CO_2インキュベーター内で培養してウイルスを細胞へ吸着させた後、1%メチルセルロースを含むEMEM 3mLを重層させ5〜7日培養した。3.7%ホルマリンで細胞を固定後、メチレンブルーで細胞を染色し、プラークの数を計測して感染価を求めた。

C.研究結果

1.エンベロープを有するJEVは低温加圧処理には抵抗性を示し、不活化効果の減弱が認められた。室温加圧と同程度の不活化効果はないと考えられた（図2）。エンベロープのないB19は低温加圧処理でも不活化され、室温加圧処理とほぼ同定度の不活化効果が期待できた（図2）。

2. 300MPaの室温加圧処理をした血漿の第8因子活性は30%にまで低下していたが、

低温加圧処理においては、59%の活性を維持していた。第９因子、フィブリノゲン、アンチトロンビンⅢの活性は、加圧処理に対して耐性が強く、300MPa でも活性を充分に維持できることが明らかとなった（図３）

3.加圧処理により、第８因子活性が低下する理由についてより詳しく調べる目的で、フォンビルブランド因子（vWF）を含む第８因子製剤と含まない第８因子製剤に対して室温、低温条件下で高圧処理を行い、その活性の変動を調べた（図４）。室温加圧と比較し、低温加圧した第８因子製剤の方がより高い活性を保っていることが確認され、これは、血漿中の第８因子活性と同様の傾向であった。さらに vWF を含まない第８因子製剤は、vWF を含む第８因子製剤と比較して高圧処理に対して抵抗性が高いことが確認された。つまり、vWF を含まない第８因子製剤を低温条件下で300MPaで処理しても、７５％の活性を維持することが確認された。加圧処理により、第８因子を安定化する役目をもつvWF の活性が低下し、結果として第８因子活性が低下する可能性が示唆された（図４）。

４．低温条件下で300MPa 加圧処理をした時の血漿中の凝固系タンパク活性を詳細に検討した（図５）。その結果、第７因子、第１１因子、プラスミノーゲン、プロテイン C、プロテイン S の活性は充分に維持されることが確認された。しかし、第１３因子の活性は著しく低下した。また大幅な活性低下が予測されたフォンビルブランド因子は 300MPa の加圧処理を行っても 85%の活性を維持していた。

D. 考察

本研究は、高圧処理により、血液製剤を汚染しうる病原体を効率よく不活化することを目的としている。そして、製剤としての機能を充分に維持していることが必要である。タンパクが変性するのは 400MPa 以上であると言われており、300MPa という圧力は、複雑な構造を持つ病原体が不活化され、かつタンパク変性することなく機能を維持することが出来るポイントである。

今年度は、室温加圧処理と比較し、低温で加圧をした方が、血漿中の凝固系タンパクの機能がより保てることを確認した（図３、図４、図５）。加圧処理により機能を障害されやすい因子は、第８因子であり、第８因子は血中ではフォンビルブランド因子と結合して存在しており、フォンビルブランド因子自体も重合して存在しているため、結果として、非常に大きな分子構造を保って機能していると考えられる。よって、構造をもつこれらの分子は加圧処理により機能を障害しやすいことが考えられた。また、第１３因子もａ鎖とｂ鎖が二本ずつ結合した a2b2 という大きな構造をとっているため、同様な理由で加圧処理により失活しやすいと考えられる（図５）。

さらに、今年度は、室温加圧処理と同様に低温加圧処理によってもヒトパルボウイル

スB19、日本脳炎JEVが不活化されるかを検討した（図2）。エンベロープを持たないB19ウイルスは室温でも低温でも同様に不活化されることが確認できたが、エンベロープを持つ日本脳炎ウイルスは、低温で加圧処理すると抵抗性を示し、充分に不活化されないことが確認された。これは低温条件下にさらすことにより、ウイルスが安定化し、高圧処理に対して抵抗性を持つようになるためと考えられる。低温条件下での加圧処理では、日本脳炎ウイルスだけでなく、エンベロープを持つ他のウイルスも不活化できない可能性が考えられる。

本年度の研究により、加圧時の温度は低温よりも室温の方がより高い不活化効果が期待できることが明らかとなった。今後は、室温加圧条件下で、適切な添加剤等を加えることによって、複雑な構造をもつタンパク質をより安定に保てるような加圧条件を見つけることが必須であると考えられる。

高圧処理による不活化は、パスカルの原理を元にした物理的破壊であるため、メチレンブルー、ソラレン等の他の不活化法と比べて多くのメリットを持つ。つまり、均一な不活化効果が期待できるためにバリデーションが行いやすい、化合物や有機物の添加が不要であるため毒性や変異原性などの有害な影響を回避し易い、一度に多くの検体を短時間で処理できる、核酸をターゲットにしていないので安全性をクリアしやすい、等の特徴をもつ優れた方法である。

今後は、大きな構造をもつ第8因子／フォンビルブランド因子、第13因子等の機能を保ちかつ、多くのウイルスを不活化できる加圧条件を見つけることが課題である。

E.結論

食品の無菌化に実用化されている高圧処理技術を血液製剤のウイルス不活化法として応用できるかを検討した。その結果、加圧処理時の温度を低温にすることで、凝固因子等の活性低下が抑制できることが確認できた。しかし、低温条件下で加圧処理を行うと、エンベロープを有するJEVは安定化して不活化できないことが明らかとなったことから、室温加圧条件の方が血液製剤の不活化法として好ましいこと考えらる。

今後、大きな構造を持つ、第8因子／フォンビルブランド因子、第13因子の機能を保てる不活化条件を決定できれば、より安全な新しい不活化法として期待でき、血液製剤の安全性確保へ貢献できる可能性が高いと考えられる。

F. 健康危機情報

なし

G. 研究発表

1.論文発表

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96:14-19　　なし

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H. 知的所有権の取得状況

図1　加圧による圧力変化
(1min x 3 cy)

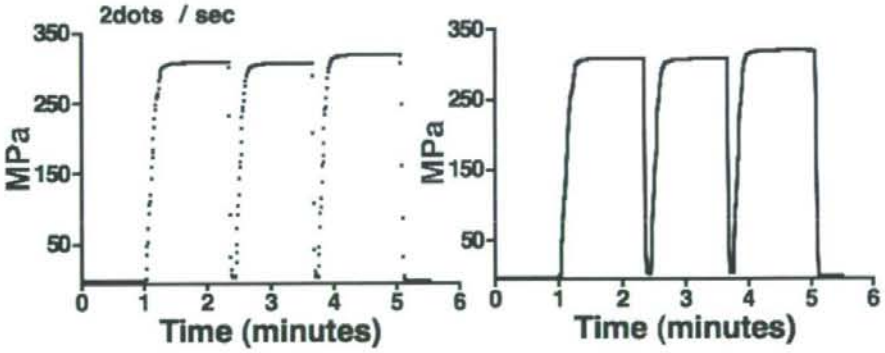


図2 低温加圧処理によるウイルス不活化

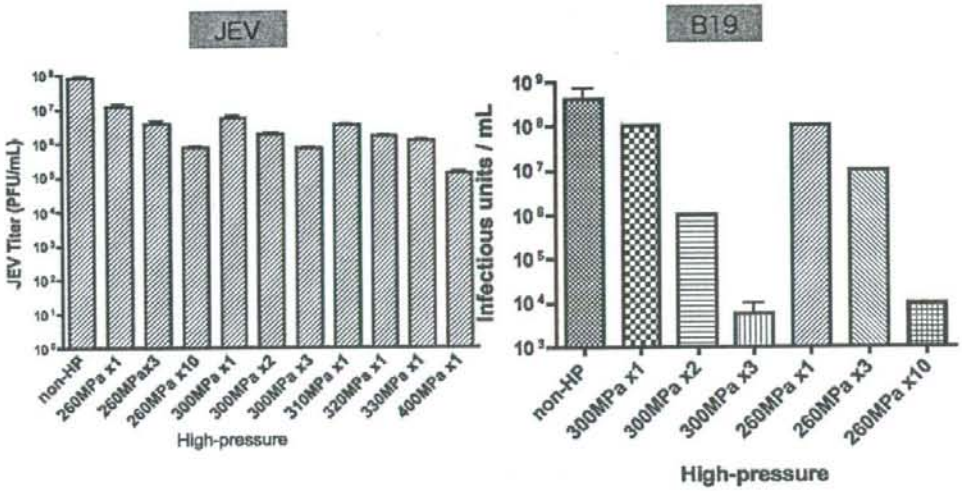


図3 低温加圧下の血漿中の凝固因子／凝固抑制因子活性

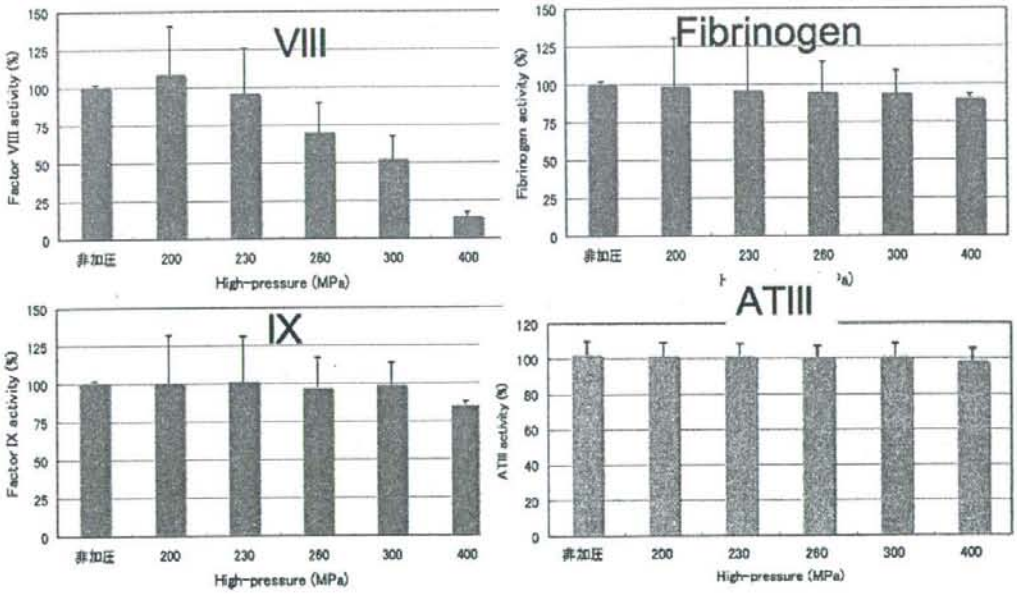


図４　低温加圧処理による第８因子製剤の活性

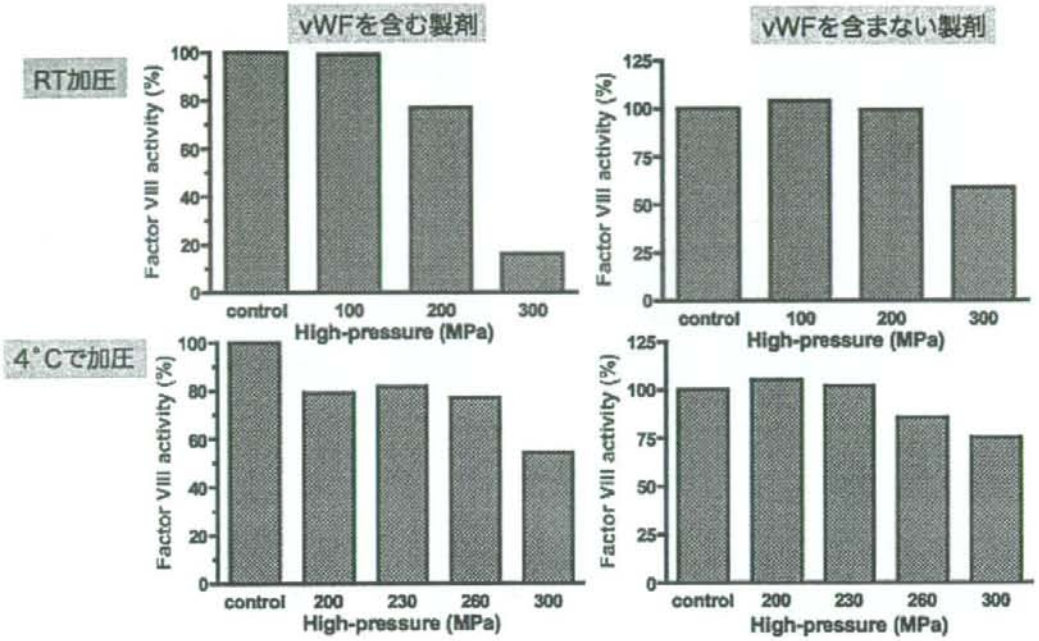
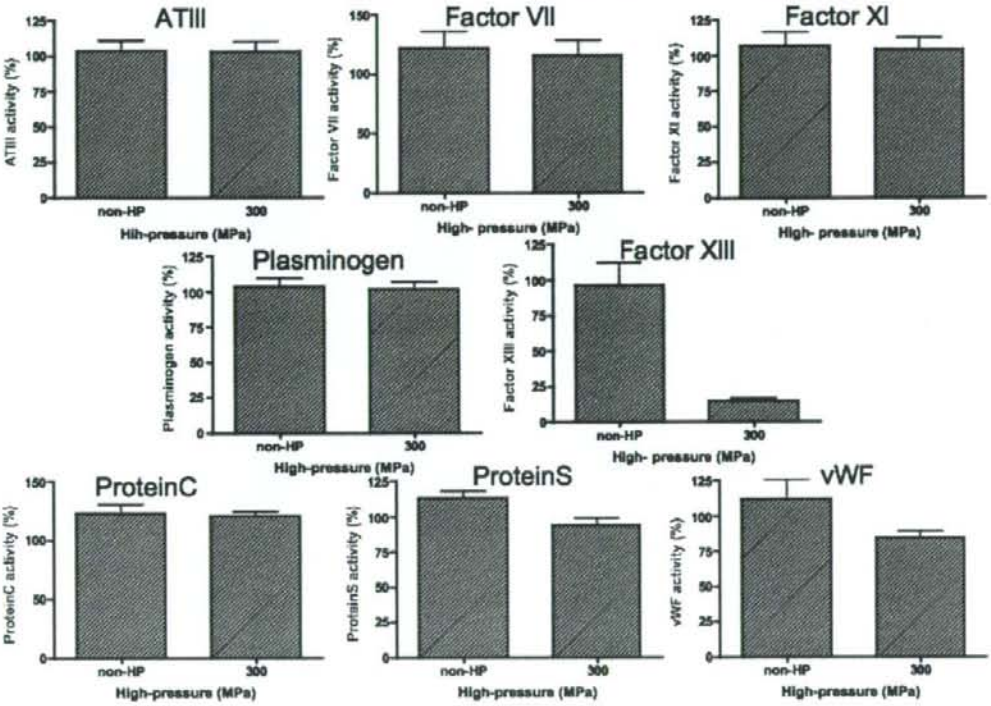


図5 低温加圧下の血漿中の凝固系タンパク活性



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
なし							

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
N.Shimazaki, T.Kiyohara, A.Totsuka,K.Nozima, Y.Okada et.al.	Inactivation of Hepatitis A virus by Heat and high hydrostatic pressure : Variation among laboratory strains	Vox Sanguinis	96	14-19	2009

IV. 研究成果の刊行物・別刷

Vox Sanguinis (2009) 96, 14–19

ORIGINAL PAPER

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Inactivation of hepatitis A virus by heat and high hydrostatic pressure: variation among laboratory strains

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Vox Sanguinis

Background and Objectives Hepatitis A virus (HAV) transmission via contaminated blood products has been reported. Cell-adapted HAV strains are generally used to confirm virus inactivation in manufacturing blood products, but the strains may differ in their sensitivity to inactivation treatment. To select an appropriate cell-adapted HAV strain for virus validation, we compared the inactivation efficiency among four strains under two different physical inactivation treatments: heat and high hydrostatic pressure.

Materials and Methods The cell-adapted HAV strains used here were KRM238, KRM003 (subgenotype IIIB), KRM031 (IA), and TKM005 (IB). The strains were treated at 60°C for up to 10 h or under high hydrostatic pressure (up to 420 MPa). The reduction in HAV infectivity was measured by an immunofocus-staining method.

Results The heat treatment at 60°C for 10 h reduced HAV infectivity in the range of 3 to 5 $\log_{10}$ among the strains; KRM238 and TKM005 were harder to inactivate than the other two. The high hydrostatic pressure treatment at 420 MPa also reduced infectivity in the range of 3 to 5 $\log_{10}$ among the strains, and KRM031 was easier to inactivate than the other strains.

Conclusion Heat treatment and high hydrostatic pressure treatment revealed differences in inactivation efficiencies among cell-adapted HAV strains, and each strain reacted differently depending on the treatment. KRM238 may be the best candidate for virus validation to ensure the safety of blood products against viral contamination, as it is harder to inactivate and it replicates better in cell culture than the other strains.

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Key words: heat inactivation, hepatitis A virus, high hydrostatic pressure inactivation, variation among strains, virus validation.

Introduction

Hepatitis A virus (HAV), which is responsible for acute viral hepatitis, is transmitted primarily by the fecal-oral route, either through the ingestion of contaminated food or water or through person-to-person contact [1,2]. On the other hand, parenteral HAV transmission has also been reported via contaminated blood [3] or blood products [4,5]. Moreover, *in vivo* HAV infection via blood reportedly has a much higher HAV infection efficiency than does oral HAV infection [6]. In developed countries such as Japan, HAV infections have become less common, owing to improved hygiene resulting from the maintenance of water and sewage facilities. Infections in early childhood are relatively rare, and thus the majority

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Table 1 Characteristics of HAV strains used

Strain	Subgenotype	Source	Year of recovery	Number of passages on African green monkey kidney cells	Titre of stock virus (FFU/ml)	Reference	Accession no.
KRM238	IIIB	Outbreak	1977	59	$1{\cdot}5 \times 10^8$	[21]	AB300205
KRM003	IIIB	Sporadic	1979	72	$1{\cdot}5 \times 10^8$	[15,18]	AB425339
KRM031	IA	Outbreak	1977	47	$1{\cdot}5 \times 10^8$	[15]	AB300206
TKM005	IB	Travel-associated	1981	48	$0{\cdot}5 \times 10^8$	[15]	AB300207

of adults remain susceptible to infection, because they lack the immunity to HAV [7]. As this could potentially facilitate massive outbreaks of hepatitis A in the general population, treatment to inactivate HAV in blood and blood products should be improved.

Previous results have demonstrated that, because HAV is a non-enveloped virus, it is quite resistant against chemical inactivation approaches, such as solvent/detergent treatments used in the preparation of blood products [8]. HAV can be inactivated however by pasteurization [9], γ-irradiation [10], and short wavelength ultraviolet light irradiation [11].

Because environmental HAV strains that have just isolated from human generally grow poorly in cell culture, cell-adapted HAV strains are generally used to test virus inactivation. As extensive genetic variation is found among cell-adapted strains [12], the strains may differ in their sensitivity to inactivation treatments. But no studies have considered the variation among cell-adapted HAV strains in testing the efficiency of inactivation treatments.

HAV strains recovered from different parts of the world have been classified into six genotypes (I–VI). Genotypes I, II and III are found in humans, and each of them is further divided into subgenotypes A and B. Most human HAV strains belong to genotypes I and III [13–15]. Subgenotype IA appears to be the predominant virus of hepatitis A cases worldwide, whereas subgenotypes IB and IIIA have been found in Scandinavia and in the Mediterranean region [16,17]. Subgenotype IIIB is unique to Japan [15,18].

To select an appropriate HAV laboratory strain for use in virus validation, we compared the rates of inactivation efficiency among cell-adapted HAV strains by using two different physical inactivation treatments – heat treatment at 60°C and high hydrostatic pressure treatment – among four cell-adapted HAV strains belonging to three subgenotypes. Heat treatment was used as a conventional inactivation treatment for blood products. High hydrostatic pressure treatment is a promising new virus-inactivating technique that is applicable to human immunodeficiency virus in blood products [19] and has been applied to HAV in food [20]. It is expected to be useful for inactivating a broad range of micro-organisms in blood products under conditions without applying high temperatures.

Materials and methods

Virus strains and propagation

Four laboratory HAV strains (KRM238, KRM003, KRM031, and TKM005) were isolated from patients with hepatitis A in Japan [15,21], and these strains were adapted by numerous passages on African green monkey kidney cells. Table 1 shows each strain's subgenotype, passage history, and stock virus titre. All four strains were propagated on an established African green monkey kidney cell line, GL37 [18].

GL37 cells were grown in Eagle's minimum essential medium supplemented with 10% fetal bovine serum (FBS) and 50 μg/ml gentamycine. To prepare the virus stocks, GL37 cells were infected at a multiplicity of infection of 0·1 focus forming units (FFU) per cell in Eagle's minimum essential medium containing 2% FBS, and were incubated for 2 weeks at 36·5°C in the presence of 5% CO_2. The infected cells were harvested by replacing the medium with phosphate-buffered saline containing 2% FBS. Virus stocks were obtained as supernatants of centrifugation at 2380 *g* for 5 min after release of the viruses by three freeze-thaw cycles and sonication of infected cells. The virus stocks were then stored at −80°C until use.

Infectivity assay

The infectious titre of each HAV strain was measured by the immunofocus-staining method described previously [21]. Briefly, a 100 μl portion of the virus dilution was inoculated into duplicate GL37 cells cultures in six-well plates at 36·5°C in the presence of 5% CO_2. After 60 min adsorption, 5 ml of the medium containing 0·6% agarose and 2% FBS was overlaid on each well. The plates were incubated at 36·5°C in the presence of 5% CO_2 for 9 days. The cells were fixed with 80% methanol containing 0·03% H_2O_2 after removal of the agarose medium. HAV foci were revealed by anti-HAV rabbit serum and horseradish peroxidase-conjugated anti-rabbit immunoglobulin G (IgG) (MBL, Nagoya, Japan) followed by colour development with DAB substrate solution (0·5 mg/ml diaminobenzidine, 0·03% $(NH_4)_2Ni(SO_4)_2$, 0·03% $CoCl_2$, and 0·03% H_2O_2 in phosphate-buffered saline).

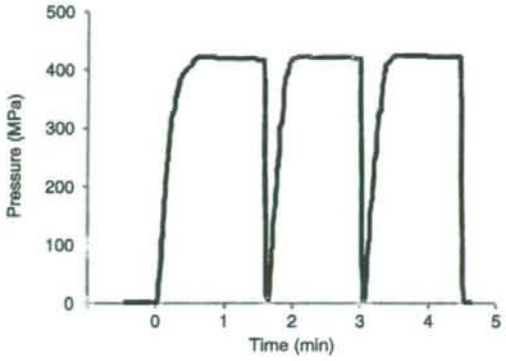

Fig. 1 The pattern of pressure change with high hydrostatic pressure at 420 MPa. Samples were treated at 25–30°C by three cycles of pressurization at the indicated pressure for 1 min followed by immediate release of the pressure. Essentially similar patterns were obtained at other hydrostatic pressures.

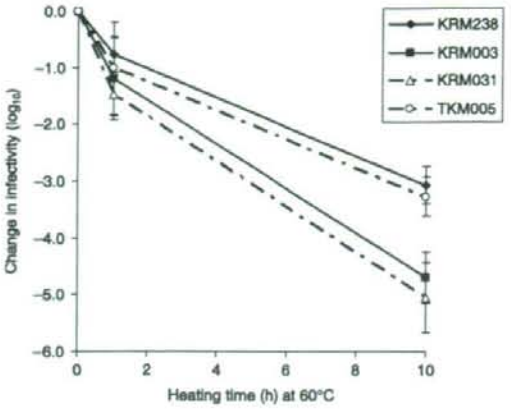

Fig. 2 Inactivation of HAV strains by heat at 60°C. The cell-adapted strains in 25% human serum albumin were treated by heat at 60°C for the indicated times. Data are the means of two or three replicates. Error bars represent the 95% confidence intervals. Change in infectivity (log_{10}) = log_{10} (titre of treated samples) − log_{10} (titre of untreated samples).

Heat treatment

The samples used for the heat treatment were prepared by adding one volume of each virus stock to 9 volumes of 25% human serum albumin (Benesis Corporation, Osaka, Japan). The samples were divided into microcentrifuge tubes in amounts of approximately 0·8 ml, and the tubes were sealed. The samples were heated at 60°C for 1 or 10 h and were then cooled on ice rapidly to arrest the heating process.

Two or three independent trials were conducted for all samples. The 95% confidence limits of these data were statistically determined and assessed; the difference was significant if it was over the 95% confidence limits.

High hydrostatic pressure treatment

The samples used for the high hydrostatic pressure treatment were prepared by adding one volume of each virus stock to 9 volumes of 5% human serum albumin. The samples were divided into ultra-centrifuge tubes (Beckman Coulter, Fullerton, CA, USA) in amounts of approximately 1·5 ml, and the tubes were sealed. The sealed tubes were placed in the chamber of a laboratory-sized high hydrostatic pressure instrument designed for food processing (Echigo Seika, Co., Ltd, Niigata, Japan). High hydrostatic pressure was controlled by water filled in the chamber. The samples were treated at 25–30°C by repeating three cycles of pressurization at the indicated pressure for 1 min and then immediately releasing the pressure. Three different pressures (300, 350, or 420 MPa) were used. At 420 MPa, the pattern of pressure change with treatment is shown in Fig. 1.

Two or three independent trials were conducted for all samples. The 95% confidence limits of these data were statistically determined and assessed; the difference was significant if it was over the 95% confidence limits.

Results

Inactivation by heat treatment at 60°C

The four cell-adapted HAV strains were treated in 25% human serum albumin with heat at 60°C for 1 or 10 h. The infectious titres of HAV in the samples were measured after heat treatment, and the reduction in HAV infectivity was then calculated. For all four strains, infectivity was reduced by approximately 1 log_{10} after heat treatment at 60°C for 1 h, indicating that HAV was resistant to heat inactivation as compared, for example, to poliovirus, which Barrett *et al.* reported was much more thermolabile than HAV [22].

With heat treatment at 60°C for 10 h, the reduction of HAV infectivity ranged from approximately 3 to 5 log_{10} among the four strains, as shown in Fig. 2. The reduction in the infectivity of KRM238 was 3·1 log_{10}, that of KRM003 was 4·7 log_{10}, that of KRM031 was 5·1 log_{10}, and that of TKM005 was 3·3 log_{10}. In other words, two strains (KRM238 and TKM005) were more resistant to inactivation by heat treatment than the other two (KRM003 and KRM031). There was 2·0 log_{10} difference between the most resistant strain KRM238 and the most sensitive strain KRM031. There was 1·6 log_{10} of variation in the inactivation rate between KRM238 and KRM003, even though they belong to the same IIIB strain subgenotype. These differences mentioned here were significant.

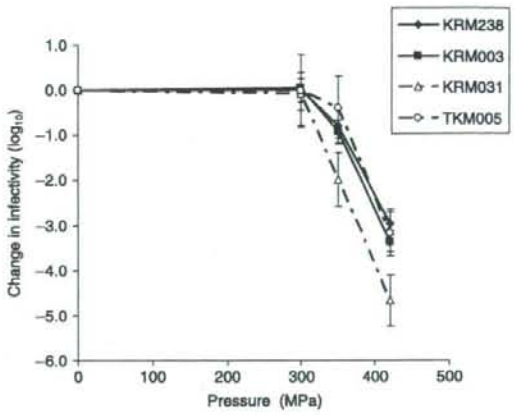

Fig. 3 Inactivation of HAV strains by high hydrostatic pressure. The cell-adapted strains in 5% human serum albumin were treated at the indicated pressures by repeating three cycles. Data are the means of two or three replicates. Error bars represent the 95% confidence intervals. Change in infectivity (log_{10}) = log_{10} (titre of treated samples) − log_{10} (titre of untreated samples).

Inactivation by high hydrostatic pressure treatment

The four cell-adapted HAV strains were treated in 5% human serum albumin with high hydrostatic pressure at 300, 350, or 420 MPa. The infectious titres of HAV in the samples were measured after the treatment, and the reduction in HAV infectivity was then calculated.

None of the HAV strains were inactivated by high hydrostatic pressure of less than 300 MPa, but all of the strains began to show inactivation at pressures exceeding 300 MPa. At 420 MPa, the reduction of HAV infectivity ranged from approximately 3 to 5 log_{10} among the strains, as shown in Fig. 3. The reduction in the infectivity of KRM238 was 3·0 log_{10}, that of KRM003 was 3·4 log_{10}, that of KRM031 was 4·7 log_{10}, and that of TKM005 was 3·2 log_{10}. There was at least 1·3 log_{10} difference, which was significant, between the resistant strains and the sensitive strain KRM031. In other words, high hydrostatic pressure inactivation was more effective against KRM031 than against the other three strains. As with heat inactivation, high hydrostatic pressure inactivation showed variation among the strains.

Accumulative effects of inactivation by heat and pressurization

To evaluate efficiency of two such inactivation treatments in the manufacture of blood products, the combined effects of inactivation by heat at 60°C for 10 h and by high hydrostatic pressure at 420 MPa are calculated by addition as shown in Table 2.

With either treatment, the degree of variation in infectivity reduction between resistant and sensitive strains was approximately 2 log_{10}. KRM238 and TKM005 well resisted inactivation by either heat or high hydrostatic pressure.

The combined reduction in the infectivity of KRM238 was 6·1 log_{10}, that of KRM003 was 8·1 log_{10}, that of KRM031 was 9·8 log_{10}, and that of TKM005 was 6·5 log_{10}.

Discussion

Cell-adapted strains are useful in studies aimed at validating the virus-inactivation procedures used in manufacturing. We report here on variation in inactivation rates – whether by heat treatment or high hydrostatic pressure treatment – among laboratory HAV strains. As shown in Table 2, if both inactivation treatments could be combined, the variation between resistant and sensitive strains would increase. For example, the most sensitive strain, KRM031, showed an estimated total reduction of 9·8 log_{10} via the combined treatments; on the other hand, the most resistant strain, KRM238, showed only a 6·1 log_{10} reduction. The maximum variation among the HAV strains after combined treatment inactivation was predicted to be about 3·7 log_{10}. To ensure the safety of

Table 2 Inactivation among HAV strains by heat and pressurization

	Reduction in infectivity (log_{10})		
HAV strain	By heat at 60°C for 10 h	By high hydrostatic pressure at 420 MPa	By combination[b] of heat and high hydrostatic pressure
KRM238	3·1 (± 0·32)[a]	3·0 (± 0·25)	6·1
KRM003	4·7 (± 0·45)	3·4 (± 0·22)	8·1
KRM031	5·1 (± 0·61)	4·7 (± 0·56)	9·8
TKM005	3·3 (± 0·35)	3·2 (± 0·52)	6·5

[a]Parentheses indicate 95% confidential limits.
[b]Expected values calculated by addition.

manufactured blood products, it is important to avoid overestimating HAV-inactivation rates. Thus, the HAV strain that is most resistant to inactivation treatment should be used in virus validation.

Considering that KRM238 grows better in cell culture than TKM005 (Table 1), it can be concluded that, among the four strains used here, KRM238 is the best candidate for virus-validation to ensure the safety of blood products against viral contamination. In general, the evaluation of inactivation processes will depend on the strains used for testing.

Our results also indicated that we should evaluate carefully the efficiency of inactivation by selecting an appropriate strain that is resistant to inactivation treatment, and that a strain that is resistant to one particular inactivation treatment may not always be resistant to another. Here, KRM003 was easily inactivated by heat treatment, showing a 4·7 $\log_{10}$ reduction, but was more stubborn against high hydrostatic pressure, which resulted in only a 3·4 $\log_{10}$ reduction. Indeed, when a novel inactivation treatment is applied to the manufacture of blood products to prevent viral contamination, inactivation treatment must be validated carefully. In other words, the efficiency of inactivation should be evaluated not only by using a strain that has shown resistance to the standard inactivation treatment, but also by selecting an appropriate strain that is resistant to a newer inactivation treatment. A test strain of virus validation for a newer inactivation should be selected carefully for avoiding a risk of overestimating the resistance of the test strain to a newer inactivation.

Pressurization has emerged as a new technique for inactivating pathogenic viruses in blood plasma and plasma-derived products, as pressurization at 400 MPa exerted no effect on the recovery of biologically active plasma proteins, with the exception of factor XIII [19]. Most enveloped viruses are markedly inactivated at pressures below 400 MPa, as summarized by Grove *et al.* [23]. However, small RNA viruses can vary widely in their sensitivity to high pressure. For example, HAV and poliovirus are both members of the picornavirus family, but they exhibit quite different susceptibilities. HAV is inactivated by 3–5 $\log_{10}$ of infectivity at 420 MPa, whereas poliovirus remains essentially unaffected even at 600 MPa [24]. At this point in time, the mechanism underlying virus inactivation by pressurization is still poorly understood.

Heat inactivation is currently used to inactivate enveloped viruses in particular, such as human immunodeficiency virus, hepatitis B virus and hepatitis C virus, in blood products. Moreover, non-enveloped viruses such as HAV and poliovirus differ greatly in terms of their sensitivity to heat inactivation [22]. As with pressurization, in heat treatment the mechanism underlying inactivation of non-enveloped viruses remains unclear.

The cell-adapted HAV strains exhibited disparate sensitivities to the two different treatments used in this study. These findings are important in terms of ensuring safety in the manufacture of blood products. Further studies will be needed in order to validate the inactivation procedures for naturally occurring viral strains.

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