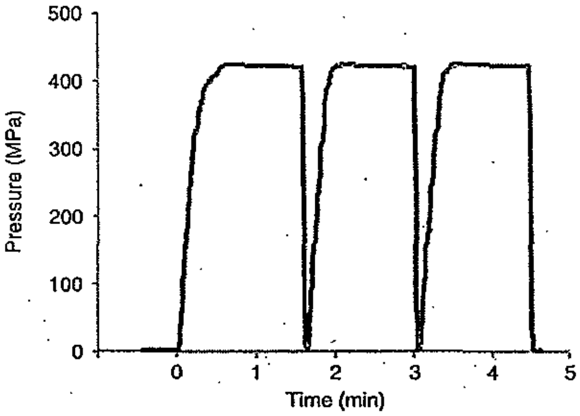

Fig. 1 The pattern of pressure change with high hydrostatic pressure at 420 MPa. Samples were treated at 25–30°C by three cycles of pressurization at the indicated pressure for 1 min followed by immediate release of the pressure. Essentially similar patterns were obtained at other hydrostatic pressures.

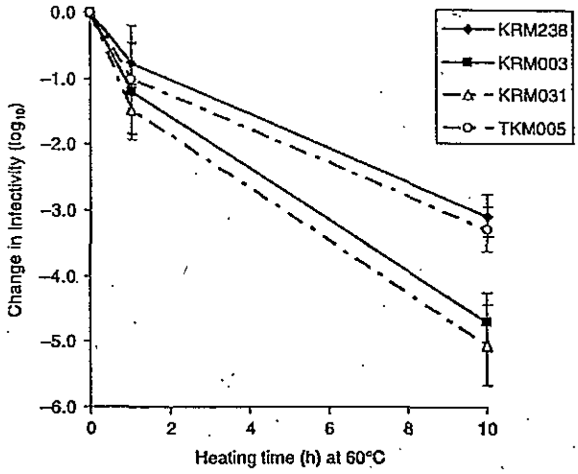

Fig. 2 Inactivation of HAV strains by heat at 60°C. The cell-adapted strains in 25% human serum albumin were treated by heat at 60°C for the indicated times. Data are the means of two or three replicates. Error bars represent the 95% confidence intervals. Change in infectivity (log_{10}) = log_{10} (titre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

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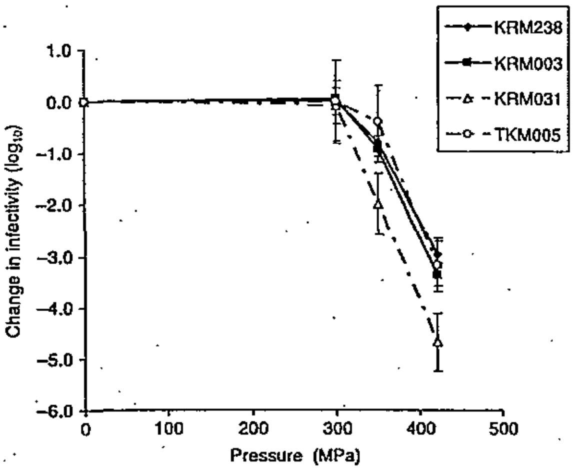

Fig. 3 Inactivation of HAV strains by high hydrostatic pressure. The cell-adapted strains in 5% human serum albumin were treated at the indicated pressures by repeating three cycles. Data are the means of two or three replicates. Error bars represent the 95% confidence intervals. Change in infectivity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

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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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医薬品　研究報告　調査報告書

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一般的名称	赤血球、血小板		研究報告の公表状況	Transfusion medicine (Oxford, England) (England) Dec 2008, 18 (6) p379-81		公表国	
販売名（企業名）	－					英国	

研究報告の概要	使用上の注意記載状況・その他参考事項等
2006 年 11 月、大阪赤十字社血液センターにおいて、繰り返し供血していた 69 歳の女性が 20-NAT で HBV DNA 陽性であることが判明し、ルーチンの検査では HBsAg、抗 HBs 抗体と抗 HBc 抗体は陰性で、EIA 法による抗 HBc 抗体だけが陽性であり、供血者が抗 HBc 抗体低値の不顕性 HBV 感染者であることを示していた。 この供血者の凍結標本を調査したところ、1999 年 10 月 1 日以降に供血された血清が個別 (ID)-NAT で HBV DNA 陽性であり、13 の供血のうち、11 が輸血に使用されていた。 受血者の HBV 検査記録を収集したが、4 例は既に原疾患で死亡しており、2 例は記録がなかった。残りの 5 例のうち 3 例では輸血前後で、2 例では輸血後のみ HBV 検査が行われており、HBV 感染のサインを示唆する情報はなかったが、HBV 感染が起きたかどうか決定するには不十分である。 神奈川赤十字社血液センターは、繰り返し血小板を提供していた ID-NAT の検出限界付近でウイルス量が揺れ動いていた不顕性 HBV 感染症の症例からの 200mL の血漿を含む濃厚血小板液での HBV 感染症を報告している。日赤血液センターによる最近のルックバック研究では、不顕性 HBV 陽性の供血者から得られた 33 の血液成分中の 1 つ (450mL の新鮮凍結血漿の輸注) で HBV 感染症を起こしており、ミニプール NAT のウインドピリオドの間に供血された 22 の血液成分中 11 の輸注での HBV 感染を明らかにした。 不顕性 HBV 感染者から血液成分の潜在的な危険を明確にするためには、さらに多くの症例が詳細に分析される必要があり、血液成分中の HBV の総輸注量、HBV 免疫抗体の保有状態、受血者の免疫状況、HBV 遺伝子型そして／あるいは突然変異の存在は算定されるべきである。	重要な基本的注意 (1) 本剤の原材料となる（献血者の）血液については、HBs 抗原、抗 HCV 抗体、・・・・陰性で、かつ ALT (GPT) 値でスクリーニングを実施している。さらに、プールした試験血漿については、HIV-1、HBV 及び HCV について核酸増幅検査 (NAT) を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。

報告企業の意見	今後の対応
不顕性 HBV 感染者 (HBsAg 陰性) からの輸血による B 型肝炎感染に関する報告である。 当社血漿分画製剤の製造工程における HBV のモデルウイルスに対するウイルスクリアランス指数は 9 以上である。なお、原料血漿はミニプール血漿における NAT 検査で HBV DNA 陰性を確認しており、最終製品においても HBV DNA 陰性を確認している。	今後ともに B 型肝炎ウイルス感染に関する安全性情報に留意していく。

②