表１．　表面元素組成（atom％）（XPS 分析結果）

		C	Cl	O	Si	Zn
未滅菌	n=1	70.6	2.3	15.1	12.0	<0.1
	n=2	73.0	1.8	14.3	10.9	0.1
	n=3	72.2	1.8	14.6	11.3	<0.1
平均値（標準偏差）		71.9 (1.22)	2.0 (0.29)	14.7 (0.40)	11.4 (0.56)	－
γ線滅菌	n=1	71.3	2.0	14.9	11.9	－
	n=2	71.7	1.5	15.1	11.7	－
	n=3	71.6	1.6	14.9	11.9	－
平均値（標準偏差）		71.5 (0.20)	1.7 (0.26)	15.0 (0.12)	11.8 (0.12)	－
EOG 滅菌	n=1	72.3	1.8	14.9	10.9	<0.1
	n=2	72.0	2.0	14.6	11.4	0.1
	n=3	71.8	2.3	14.3	11.4	0.1
平均値（標準偏差）		72.0 (0.25)	2.0 (0.25)	14.6 (0.30)	11.2 (0.29)	－
高圧蒸気滅菌	n=1	71.4	2.2	14.8	11.7	－
	n=2	71.4	2.2	14.5	11.9	－
	n=3	71.1	2.1	14.5	12.2	－
平均値（標準偏差）		71.3 (0.17)	2.2 (0.06)	14.6 (0.17)	11.9 (0.25)	－

－：検出限界以下

表 2.　3 次元表面粗さ評価結果（観察視野 2mm×2mm）

試料	Ra(μm)	Rq(μm)	Rmax(μm)
未滅菌	3.93	5.36	54.6
γ線滅菌	4.04	5.46	51.6
EOG 滅菌	4.28	5.78	56.8
高圧蒸気滅菌	4.61	5.97	48.8

・Ra（算術平均粗さ）　：定量面で中心面から表面までの偏差の絶対値の平均値
・Rq（二乗平均粗さ）　：定量面で中心面から表面までの偏差の二乗の平均値の平方根
・Rmax（最大最小粗さ）　：定量面で最も高い山から最も低い谷間での垂直距離

※Rmax は、像内にたまたま入った高い突起や深い穴の影響を直接受けるため、ばらつきが大きく、試料間の精密な比較には向いていない。

表 3.　表面付近硬さ評価結果（ナノインデンター分析結果）

試料	押し込み深さ約 1μm		押し込み深さ約 5μm		押し込み深さ約 20μm	
	弾性率 ／ MPa	硬さ ／ MPa	弾性率 ／ MPa	硬さ ／ MPa	弾性率 ／ MPa	硬さ ／ MPa
未滅菌	110 (24)	0.80 (0.30)	61 (14)	0.78 (0.37)	58 (8)	1.1 (0.2)
γ線滅菌	290 (77)	4.9 (1.4)	83 (26)	2.1 (0.53)	42 (22)	0.96 (0.38)
EOG 滅菌	100 (32)	0.74 (0.31)	47 (11)	0.47 (0.34)	40 (11)	0.66 (0.10)
高圧蒸気滅菌	85 (18)	0.39 (0.11)	40 (5)	0.19 (0.03)	39 (6)	0.52 (0.09)

・表中の数値は、測定 n 数 8～11 の平均値

・( ) の数値は標準偏差

表 4.　深さ方向別 DEHP 含有量（切削－熱脱着 GC/MS 分析結果）

試料名	部位	平均切削厚み	DEHP 量
未滅菌	全体	－	32.1Wt%（別途定量値）
	表層～65μm	65μm	32.5Wt%
	内層(65～108μm)	43μm	32.9Wt%
γ線滅菌	全体	－	27.8Wt%（別途定量値）
	表層～56μm	56μm	30.4Wt%
	内層(56～116μm)	60μm	31.9Wt%
EOG 滅菌	全体	－	26.8Wt%（別途定量値）
	表層～45μm	45μm	30.7Wt%
	内層(45～103μm)	58μm	32.0Wt%
高圧蒸気滅菌	全体	－	26.8Wt%（別途定量値）
	表層～48μm	48μm	31.3Wt%
	内層(48～105μm)	57μm	35.8Wt%

Ⅲ. 分担研究報告書

4. ポリカーボネート製三方活栓の医薬品によるひび割れの分析

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プラスティック製医療用具の適正使用に関する研究

分担研究報告書
ポリカーボネート製三方活栓の医薬品によるひび割れの分析

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研究要旨

複数の医薬品を輸液する際に使用されるポリカーボネート製三方活栓は、使用中にまれに、メスコネクタ部にひび割れが発生することが報告されているが、発生の条件については十分解明されていなかった。

本研究において医薬品の添加剤成分、三方活栓に接続する医療用具と三方活栓との接合強度、接合回数及び接合時間の条件などを広範囲に検討した結果、三方活栓のひび割れの発生条件及び発生傾向が明らかになった。

A.研究目的

ポリカーボネート樹脂は透明であり、耐衝撃性、耐熱性が高く、成形での寸法精度が高いことから様々な医療用具に使用されており、市販されている三方活栓は殆どがポリカーボネート製である。

一方、ポリカーボネート製の三方活栓は使用中にひび割れが生じることが知られており、中尾ら[1]はひび割れの原因が脂肪乳剤によることを報告していたが、脂肪乳剤以外の医薬品添加剤でもひび割れが発生することを医療機関等から指摘を受けたことから原因をより広範囲に追求することとした。

本研究では、三方活栓のひび割れの発生について、医薬品の添加剤の種類と濃度、三方活栓と三方活栓に接続する医療用具との接続の締め付け強度、回数などによるひび割れの発生傾向を分析した。

B.研究方法

本研究では、ひび割れの発生条件として医薬品の添加剤の種類の他、三方活栓と他の医療用具との締め付け強度（強さ）、締め付けの回数、締め付けた状態での時間（使用時間を想定）を設定し試験を実施した。

1）医薬品の添加剤の種類及び濃度

脂肪乳剤、ポリオキシエチレン硬化ヒマシ油、ポリソルベート、プロピレングリコール、エチレンジアミン、ベンジルアルコールを添加剤成分として含む医薬品について試験した。脂肪乳剤については脂肪乳剤の含有濃度が20%と10%について試験した。

対照として生理食塩液を使用した。全ての医薬品は原液のまま使用した。

2）締め付け強度

締め付け強度は、臨床使用において一般的な締め付けの強度を想定した15cN·m（cN·m；センチニュートン・メートル）、男性が強く締め付けた場合

を想定した 30cN・m、更に、過酷に締め付けを行った場合を想定した 40cN·m の 3 種類で締め付けした。

3) 締め付けの回数

三方活栓とロック式のオスコネクタを一度締め付けた状態で所定の時間放置した方法(方法 1 と呼ぶ) と、三方活栓とロック式のオスコネクタを締め付けた後放置し、観察時間に接続を一旦外し、ひび割れを観察後に、締め直しを行い、観察時間ごとに同じ脱着を繰り返す方法 (方法 2 と呼ぶ) とした。

方法 1 は臨床現場において「三方活栓に他の医療用具のオスコネクタを接続した後、接続を外すことなく使用する場合」を想定した。また、方法 2 は「三方活栓に他の医療用具のオスコネクタを接続し、一旦使用した後、接続する他の医療用具を新しく交換し三方活栓を継続使用する場合」を想定した。

4)ひび割れの観察方法

市販の三方活栓のメスコネクタ部に医薬品を数滴滴下し、ロックタイプのオスコネクタをトルクゲージ (東日製作所製) を用いて締め付けを行った。ひび割れの確認は、三方活栓とロック式のオスコネクタを接続した状態で圧力 20kPa のエアで水没試験し、エアリークが確認されたものをひび割れありとした。初回締め付け直後、1 日後、2 日後、3 日後、4 日後及び 7 日後に観察した。

C.研究結果

C-1)脂肪乳剤を含有する医薬品

方法 1、すなわち締め直しを実施しない場合では締め付け強度、時間に関係なくひび割れは発生しなかった。

方法 2、すなわち締め直しを実施した場合では締め直し 2 回でひび割れが確認され、締め直し回数が増えるに従って、ひび割れの発生率は増加した。

脂肪乳剤の濃度によるひび割れの発生率は締め直し 5 回のひび割れ発生率は 10%の脂肪乳剤濃度が 34.8%、20%濃度が 45.2%で脂肪乳剤の濃度の高い方がひび割れを発生することが確認された。

また、締め付け強度によるひび割れの発生率の差は、脂肪乳剤濃度 10%の場合、締め直し 6 回の結果において 15cN·mが 15.6%、30cN/m が 41.1%、40cN·m が 51.2%であり、締め付け強度はひび割れに強い影響を与えることが明確になった。

C-2)その他の添加剤

添加剤としてポリオキシエチレン硬化ヒマシ油、ポリソルベート、ベンジルアルコールを用いたときには締めなおしを実施しない場合には脂肪乳剤と同様、ひび割れは発生しなかった。締め直しを実施した場合はいずれの添加剤も脂肪乳剤と同様な傾向を示したが、その発生率の大雑把な比較ではベンジルアルコール＞ポリソルベート＞ポリオキシエチレン硬化ヒマシ油であった。

添加剤としてプロピレングリコールとエチレンジアミン両方を含有する医薬品を用いた場合には締め直しなしであっても締め付け強度 40cN·m では 1 日後であってもひび割れが確認され 7 日後の発生率は 55.7%という高い発生率を示した。

一方、対象として選ばれた生理食塩液では方法 1 及び方法 2 ともに締め付け強度、締め直し回数、時間に関係なくにひび割れは一切、発生しなかった。

D.考察

今回の試験では、発生原因を追求する目的で添加剤などの医薬品は原液を使用したので、実験で得られたひび割れの発生率は実際の臨床現場において発生するひび割れの頻度をはるかに超える数値であったが、発生傾向などは明確になったと考えられる。

1) 添加剤として汎用される脂肪乳剤、ポリソルベ

ート、ベンジルアルコール等では締め直しを実施しない場合は 7 日間という長期間であってもひび割れは発生しなかったが、締め直しを実施した場合はひび割れが発生しやすいことが判明した。臨床現場では接続部の外れ、液漏れを防ぐ目的でしばしば、締め直しをすることが観察されていることから、それに対しての十分な対策が必要である。

2)、プロピレングリコールとエチレンジアミン両方を含有する医薬品では締め直しを行わない場合でもひび割れが確認されたことから、脂肪乳剤等よりも、更に強くひび割れを誘引する可能性がある。

添加剤としてプロピレングリコールとエチレンジアミン両方を含有する医薬品が臨床現場でどのような条件で使用されているか、例えば、薬剤濃度や使用期間など、更に詳細な調査をする必要があると思われる。

結論　ひび割れの発生には次の傾向があった。

- 締め付け強度が強いほど発生率は高くなる。
- 締め直し回数が多いほど発生率は高くなる。
- 締め付けた状態での使用時間が長いほど発生率は高くなる。
- 医薬品の添加剤濃度が高いほど発生率は高くなる。

三方活栓のひび割れの発生のリスクを低減する対策として、①ひび割れを生じる可能性が高い医薬品を使用する場合には三方活栓に接続する医療用具との強い締め付けを行わないこと、②締め直し回数を減らすこと、③医薬品の濃度についても考慮することの対応が必要である。

参考文献

1)　「中尾正和他、プロポフォール使用時の三方活栓ひび割れ減少への薬物の影響」麻酔 49 巻（第 7 号）、802-805,1998 年

Table 1　締め付け強度 15cN·m で繰り返し締め付けを行わない（方法 1）条件でのひび割れの発生率

経過時間 医薬品の添加剤成分（濃度）	1 時間	1 日	2 日	3 日	4 日	7 日
脂肪乳剤(20%)	0.0	0.0	0.0	0.0	0.0	0.0
脂肪乳剤(10%)	0.0	0.0	0.0	0.0	0.0	0.0
ポリオキシエチレン硬化ヒマシ油	0.0	0.0	0.0	0.0	0.0	0.0
ポリソルベート	0.0	0.0	0.0	0.0	0.0	0.0
プロピレングリコール及びエチレンジアミン	0.0	0.0	0.0	0.0	0.0	4.3
ベンジルアルコール	0.0	0.0	0.0	0.0	0.0	0.0
生理食塩液	0.0	0.0	0.0	0.0	0.0	0.0

Table2　締め付け強度 40cN·m で繰り返し締め付けを行わない（方法 1）条件でのひび割れの発生率

経過時間 医薬品の添加剤成分（濃度）	1 時間	1 日	2 日	3 日	4 日	7 日
脂肪乳剤(20%)	0.0	0.0	0.0	0.0	0.0	0.0
脂肪乳剤(10%)	0.0	0.0	0.0	0.0	0.0	0.0
ポリオキシエチレン硬化ヒマシ油	0.0	0.0	0.0	0.0	0.0	0.0
ポリソルベート	0.0	0.0	0.0	0.0	0.0	0.0
プロピレングリコール及びエチレンジアミン	0.0	8.6	22.9	37.1	44.3	55.8
ベンジルアルコール	0.0	0.0	0.0	0.0	0.0	0.0
生理食塩液	0.0	0.0	0.0	0.0	0.0	0.0

Table3　締め付け強度 15cN·m で繰り返し締め付ける（方法 2）条件でのひび割れの発生率

締め直し回数 医薬品の添加剤成分（濃度）	1 回	2 回	3 回	4 回	5 回	6 回
脂肪乳剤(20%)	0.0	0.0	0.0	2.9	7.1	17.1
脂肪乳剤(10%)	0.0	0.0	1.1	4.4	10.0	17.8
ポリオキシエチレン硬化ヒマシ油	0.0	0.0	0.0	1.4	1.4	1.4
ポリソルベート	0.0	0.0	0.0	0.0	0.0	3.3
プロピレングリコール及びエチレンジアミン	0.0	1.4	1.4	5.7	8.6	12.9
ベンジルアルコール	0.0	0.0	5.0	5.0	10.0	15.0
生理食塩液	0.0	0.0	0.0	0.0	0.0	0.0

Table4　締め付け強度 40cN·m で繰り返し締め付ける（方法 2）条件でのひび割れの発生率

締め直し回数 医薬品の添加剤成分（濃度）	1 回	2 回	3 回	4 回	5 回	6 回
脂肪乳剤(20%)	0.0	0.0	17.1	32.9	41.4	60.0
脂肪乳剤(10%)	0.0	4.4	6.7	15.6	25.6	45.6
ポリオキシエチレン硬化ヒマシ油	0.0	0.0	1.4	2.9	2.9	7.1
ポリソルベート	0.0	16.7	30.0	46.7	46.7	46.7
プロピレングリコール及びエチレンジアミン	0.0	25.7	48.6	70.0	77.1	81.4
ベンジルアルコール	0.0	10.0	20.0	40.0	50.0	50.0
生理食塩液	0.0	0.0	0.0	0.0	0.0	0.0

Ⅳ. 研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル	発表雑誌	巻号	ページ	出版年
S.Takatori et al.	Determination of di(2-ethylhexyl) phthalate and mono(2-ethylhexyl) phthalate in human serum using LC/MS/MS.	J. Chromatogr. B	**804**	397-401	2004
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V. 研究成果の刊行物・別刷り

Available online at www.sciencedirect.com
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Journal of Chromatography B, 804 (2004) 397–401
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Determination of di(2-ethylhexyl)phthalate and mono(2-ethylhexyl)phthalate in human serum using liquid chromatography-tandem mass spectrometry

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Abstract

Concentrations of mono(2-ethylhexyl)phthalate (MEHP), and di(2-ethylhexyl)phthalate (DEHP), in serum of healthy volunteers were determined by high performance liquid chromatography (HPLC) with tandem mass spectrometry (LC/MS/MS). The serum was extracted with acetone, followed by hexane extraction under acidic conditions, and then applied to the LC/MS/MS. Recoveries of 20 ng/ml of MEHP and DEHP were 101 ± 5.7 (n = 6) and 102 ± 6.5% (n = 6), respectively. The limits of quantification (LOQ) of MEHP and DEHP in the method were 5.0 and 14.0 ng/ml, respectively. The concentration of MEHP in the serum was at or less than the LOQ. The concentration of DEHP in the serum was less than the LOQ. Contaminations of MEHP and DEHP from experimental reagents, apparatus and air during the procedure were less than the LOQ and were estimated to be <1.0 and 2.2 ± 0.6 ng/ml, respectively. After subtraction of the contamination, the net concentrations of MEHP and DEHP in the serum were estimated at or <5 and <2 ng/ml, respectively. To decrease contamination by DEHP, the cleanup steps and the apparatus and solvent usage were minimized in the sample preparation procedures. The high selectivity of LC/MS/MS is the key for obtaining reliable experimental data from in the matrix-rich analytical samples and for maintaining a low level contamination of MEHP and DEHP in this experimental system. This method would be a useful tool for the detection of MEHP and DEHP in serum.

Keywords: Di(2-ethylhexyl)phthalate; Mono(2-ethylhexyl)phthalate

1. Introduction

Di(2-ethylhexyl)phthalate (DEHP) is a common plasticizer used to impart flexibility to polyvinylchloride (PVC). It leaches readily from PVC into the environment and transfers to other materials attached to the PVC or via the atmosphere. Patients undergoing medical procedures, such as intravenous therapy, nutritional support, blood transfusion, hemodialysis, cardiopulmonary bypass or extracorporeal membrane oxygenation (EMO) can be exposed to DEHP. Previous studies have shown detectable amounts of DEHP in blood products, in intravenous solutions, and in intravenous fat emulsions stored in PVC bags [1–5]. In animal studies, DEHP and/or MEHP are toxicants to the reproductive and developmental systems [6–10]. DEHP is hydrolyzed to MEHP in vivo and in blood products by esterase activities [11,12]. DEHP and MEHP have been detected in the blood of hemodialysed patients [13,14]. The FDA Center for Devices and Radiological Health (FDA/CDRH) has reviewed the potential health risks of DEHP leaching from medical devices [15]. Furthermore, the FDA/CDRH has recommended considering alternatives when high-risk procedures including transfusion, hemodialysis, total parenteral nutrition, EMO, or enteral nutrition are to be performed on male neonates, pregnant women who are carrying a male fetus, and peripubertal males [16].

To assess patient risk of DEHP and MEHP intake via medical procedures, the concentration of DEHP and MEHP in drugs, blood products and patients' serum should be determined accurately. However, the widespread usage and stability of DEHP in the experimental environment have led to DEHP being present as a ubiquitous contaminant. For this reason, the contamination of DEHP arising from

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the environment often injures the reliability of experimental data. There are documented cases of high levels of DEHP contamination in experimental environments and/or including reagents in DEHP measurements [17,18]. To decrease contamination by DEHP, it is reasonable to minimize the cleanup steps, and the apparatus and solvent usage. However, omission of the cleanup steps increases the potential for enough matrices remaining in the analytical samples to interfere with the accurate determination of analytes. To overcome this problem, we have adopted a high performance liquid chromatography with tandem mass spectrometry (LC/MS/MS) system for its high selectivity of the analytes. High performance liquid chromatography (HPLC) systems can measure MEHP without esterification at the carboxylic group of MEHP. Furthermore, elution performed in an isocratic mode is free from detection of MEHP and DEHP from in the LC systems including pump, lines, ferrules and eluents. These are advantages of HPLC systems over gas chromatography systems. Here, we describe a simple and sensitive method for determination of the concentrations of MEHP and DEHP in human serum by using LC/MS/MS.

2. Experimental

2.1. Materials

DEHP (99.6%), MEHP (99.3%), DEHP-d4 (99.0%) and MEHP-d4 (99.8%) were purchased from Hayashi Pure Chemical Industries Ltd. (Osaka, Japan). Environment analytical grade acetone, hexane and acetonitrile were obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan). HPLC grade acetonitrile and acetic acid were also obtained from Wako Pure Chemical Industries Ltd. The water for HPLC was purified by the Milli-Q system (Milli-Q, Millipore, Saint-Quentin Yvelines, France). The water for extraction was prepared by washing the Milli-Q water with hexane.

To eliminate contamination of DEHP and MEHP from glassware, the glassware was washed twice with acetone and hexane and then baked at 200 °C for 2 h in a clean oven.

2.2. Preparation of standard solutions and human serum

The stock solutions of DEHP, MEHP, and their isotopes were prepared in acetonitrile at 1.0 mg/ml using DEHP and MEHP-free glassware. They were mixed at the desired ratio and serially diluted for calibration curves. Human blood was obtained from four healthy volunteers with syringes made of glass through metal needles. To prepare serum samples, the blood was allowed to stand at 20 °C for 30 min in glass tubes with aluminum foil caps and then centrifuged at 3000 rpm for 10 min. The serum was stored at −40 °C until analysis. To avoid the contamination of DEHP and MEHP, all glassware and metal needles were washed and baked as mentioned above. The gender, age, body weight and nutrition of the volunteers are shown in Table 1.

Table 1
The gender, age, body weight and nutrition of four volunteers (A, B, C and D)

	Gender	Age (years)	Body weight (kg)	Meal[a]	Nutrition[b]
A	F	30	56	M1	*Bread* (40 g), *butter* (3 g), apple (40 g), coffee (400 ml)
				M2	*Rice* (80 g), grilled horse mackerel, deep-fried vegetables (pumpkin, onion, asparagus, eggplant; 20 g each), soup (miso 20 g, sweet potato, radish, 20 g each)
				M3	Pasta (200 g), source (200g; ground meat, tomato, onion, potato, cheese)
				M4	*Bread* (80 g), apple (40 g), coffee (200 ml), *yogurt* (50 g)
B	M	28	63	M1	Rice balls (200g)
				M2	Beef stew (250 g; beef, onion, carrot, potato, source), *deep-fried chicken* (100 g), beer (350 ml)
				M3	Pasta (100 g), mushrooms (30 g), thick white noodles made of wheat flour, salt and water (200 g)
				M4	Bread (90 g), coffee (180 ml)
C	M	29	62	M1	Cereal (30 g), milk (100 ml), coffee (200 ml), banana (90 g)
				M2	Rice (200g), boiled chicken (150 g), *lettuce* (100g), *spinach* (50 g), *soybean pulp* (50 g), soup (miso 20 g, potato, onion, 10 g each)
				M3	*Burger put deep-fried chicken* (200 g), french fries (50 g), *deep-fried chicken* (50 g), orange juice (200ml)
				M4	*Bread* (80 g), blueberry jam (10 g), milk (100 ml)
D	M	34	58	M1	Rice (80 g), *pancake* (200 g; wheat flour, pork, cabbage, egg), soup (miso 20 g, onion 20 g), omelet (20 g)
				M2	Rice (200 g), chinese-style dumpling (300 g; wheat flour, ground meat, chinese cabbage, onion, spring onion), boiled crab (50 g)
				M3	Doughnuts (150 g), *Corn snack foods* (75 g)
				M4	Rice balls wrapped with deep-fried soybean curds (250 g)

Blood sampling was performed at 10 a.m. (set at zero time). The nutrition of the volunteers taken prior to the blood sampling for 26 h was presented.
[a] M1, taken at 3–4 h; M2, taken at 13–16 h; M3, taken at 20–22 h; M4, 24–26 h.
[b] The weight of nutrition was at served. Italicized: nutrition served in a plastic dish or a package.

2.3. Sample preparation procedures

To a tube containing 0.50 g of serum, 10 ng of MEHP-d4 and DEHP-d4 was added at 4 °C. Then, 4.0 ml acetone was added and the sample was sonicated for 2 min and vigorously shaken for 5 min. The serum was centrifuged at 3×10^3 g and the supernatant was collected. To the precipitant, 1.0 ml acetone was added and extracted as described above. The supernatants were combined together and dried under an N_2 stream. To this tube 0.50 ml hexane-washed water and 4.0 μl acetic acid were added. After a 2 min sonication, MEHP and DEHP were extracted four times with 1.0 ml hexane. After drying under an N_2 stream, the extract was resolved in 0.50 ml acetonitrile. The analytical samples were placed in inactivated insert vials capped with aluminum foil and 5.0 μl of these samples were injected into an LC/MS/MS system.

2.4. LC/MS/MS conditions

LC/MS/MS analysis was performed on an API3000 (Applied Biosystems, Foster City, CA) equipped with an electrospray ionization (ESI) interface and an Agilent 1100 series HPLC from Agilent Technologies (Waldbronn, Germany). The HPLC system consisted of a G1312A HPLC binary pump, a G1367A autosampler and a G1379A degasser. A reverse phase HPLC column (Wakosil3C18, 2.0 × 100 mm, 3 μm; Wako Pure Chemical Industries Ltd.) was used. The mobile phases consisted of 100% acetonitrile (A) and 0.05% aqueous acetic acid (B). Elution was performed using an isocratic mode (A/B: 15/85, v/v) at 0.2 ml/min. The ESI interface was controlled by the Analyst software (v.1.3.2). ESI-MS was operated in negative or positive ion mode. The heated capillary and voltage were maintained at 500 °C and ±4.0 kV (negative/positive mode), respectively. MEHP and MEHP-d4 were detected in the negative mode. DEHP and DEHP-d4 were detected in the positive mode. Daughter ion mass spectra of MEHP, MEHP-d4, DEHP and DEHP-d4 obtained by the LC/MS/MS system are shown in Fig. 1. The combinations of parent ions and daughter ions were as follows; MEHP (parent ion/daughter ion, 277/134), MEHP-d4 (281/138), DEHP (391/149), DEHP-d4 (395/153). The daughter ions were formed in the collision cell using N_2 gas as the collision gas. The optimum collision energies for MEHP (MEHP-d4) and DEHP (DEHP-d4) were −22.0 and 27.0 V, respectively.

3. Results

The retention times of MEHP, MEHP-d4, DEHP and DEHP-d4 were 3.0, 3.0, 25.6 and 25.3 min, respectively. The relative standard deviations of the retention times were <0.03%. The signal to noise ratios of the MRM (multiple reaction monitoring) of 1 ng/ml MEHP and DEHP were 4.0 and 3.5, respectively. For MEHP measurement, the calibration curve was obtained for the peak-area ratio (MEHP/MEHP-d4) versus the MEHP concentration. It was linear over the range of 2.0–500 ng/ml. The mean linear regression equations obtained from five replicates were $y = 0.0581x - 0.097$ ($r = 0.999$) with mean values for slope and intercept of 0.0581 ± 0.0012 (mean ± S.D.; S.D., standard deviation) and −0.097 ± 0.017, respectively (y, peak-area

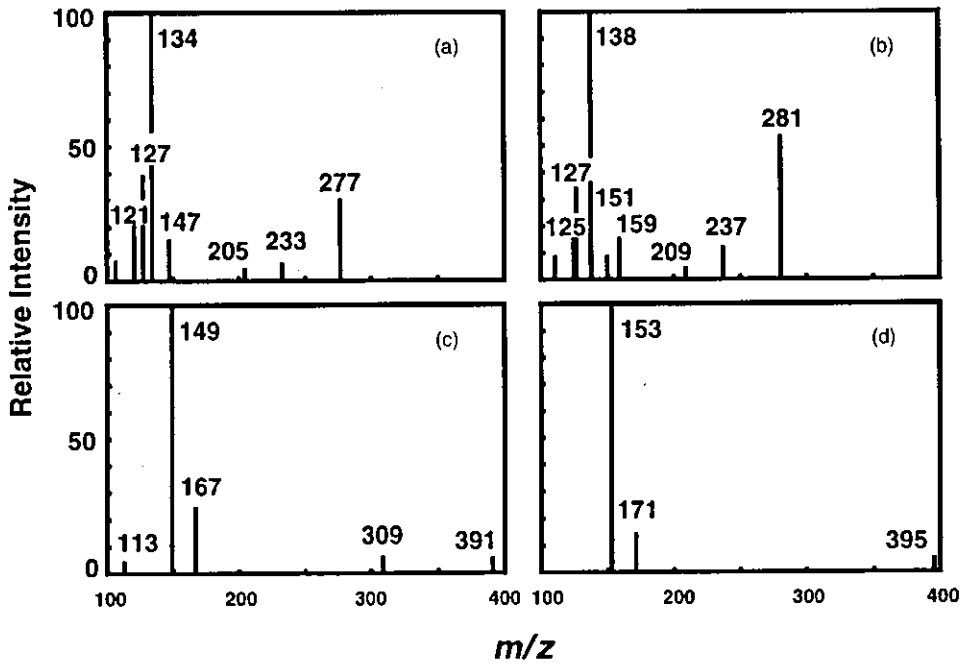

Fig. 1. Daughter ion spectra of MEHP (a), MEHP-d4 (b), DEHP (c), and DEHP-d4 (d).

Table 2
Concentrations of MEHP and DEHP in human sera

Serum from volunteers	Concentration (ng/ml)	
	MEHP[a]	DEHP[b]
A	5.7 ± 2.7	N.D. (3.8 ± 1.3)
B	N.D. (4.1 ± 1.5)	N.D. (3.7 ± 0.8)
C	N.D. (3.3 ± 0.6)	N.D. (2.9 ± 0.6)
D	N.D. (3.4 ± 0.6)	N.D. (3.9 ± 1.0)
Blank	N.D. (<1.0)	N.D. (2.2 ± 0.6)

The blank was the result of measurements of MEHP and DEHP in hexane washed water which contained >1 ng/ml MEHP and 1 ng/ml DEHP. Values in parentheses represent averages of the five independent measurements and SDs.

[a] N.D.; MEHP concentrations lower than 5 ng/ml.

[b] N.D.; DEHP concentrations lower than 14.0 ng/ml.

ratio; x, MEHP concentration ng/ml). For DEHP measurement, the calibration curve was obtained for the peak-area ratio (DEHP/DEHP-d4) versus DEHP concentration. It was linear over the range of 1.0–4000 ng/ml. The mean linear regression equations obtained from five replicates were $y = 0.0318x + 0.337$ ($r = 0.999$) with mean values for slope and intercept of 0.0318 ± 0.0012 and 0.337 ± 0.035, respectively (y, peak-area ratio; x, DEHP concentration ng/ml). The recovery tests were performed using MEHP-d4 and DEHP-d4 to avoid the effects of possible contamination by MEHP and DEHP. The recoveries of 20 ng/ml of MEHP-d4 and DEHP-d4 from human serum were 101 ± 5.7 ($n = 6$) and 102 ± 6.5% ($n = 6$), respectively. The recoveries of 100 ng/ml of MEHP-d4 and DEHP-d4 from human serum were 93.8 ± 6.8 ($n = 6$) and 102 ± 6.2% ($n = 6$), respectively.

To determine the contamination of DEHP and MEHP generated by this extraction method, a blank test was performed using hexane-washed water instead of human serum. The concentrations of MEHP and DEHP in sera of healthy volunteers and the blank are shown in Table 2. The typical MRM chromatogram of the human serum is shown in Fig. 2. The

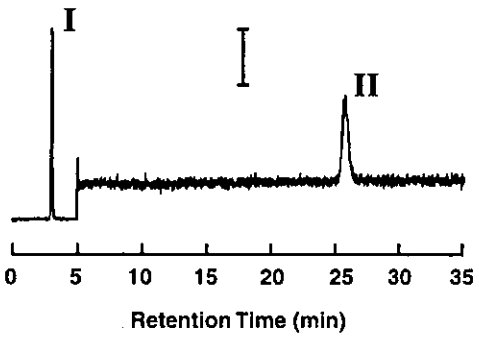

Fig. 2. The MRM chromatogram of human serum. From 0 to 5 min, monitoring of the daughter ion (m/z 134) of the parent ion (m/z 277), is in the negative mode. From 5 to 35 min, monitoring of the daughter ion (m/z 149) of the parent ion (m/z 391), is in the positive mode. The bar corresponds to 5.0×10^3 counts per second. The concentrations of MEHP (I) and DEHP (II) were estimated at 3.2 and 3.7 ng/ml, respectively.

blank of MEHP and DEHP were <1.0 and 2.2 ± 0.6 ng/ml, respectively. The limits of quantification (LOQ) of MEHP and DEHP in this method were determined by the formula, LOQ = 5 x (the blank + S.D.), and were 5 and 14.0 ng/ml, respectively. The concentrations of MEHP and DEHP in sera of healthy volunteers were at or below the LOQ. The concentrations of MEHP and DEHP under the LOQ are shown in parentheses. The concentrations include the blank levels of MEHP and DEHP. Thus, the net concentrations of MEHP and DEHP in the human serum were estimated at or <5 and <2 ng/ml, respectively.

4. Discussion

Severe contamination of MEHP and DEHP make it difficult to know the accurate concentrations of MEHP and DEHP in normal serum. In our trial to determine the MEHP in the serum by using gas chromatography with mass spectrometry, the contaminations of MEHP and DEHP derived from the esterification with 2,3,4,5,6-pentafluorobenzylbromide and subsequent clean up were 120 and 420 ng/ml, respectively (data not shown). By using an LC/MS/MS system, we developed a method to measure the concentrations of MEHP and DEHP with a low level contamination, and demonstrated that in serum of healthy volunteers these concentrations were at or less than the LOQ (5 and 14.0 ng/ml, respectively). Kessler et al. concluded that MEHP and DEHP in blood obtained from rats actually presented minute amounts, because there was no difference between the concentrations obtained from rat blood and water [17]. Inoue et al. have developed a method using LC/MS with column-switching systems for measurement of MEHP and DEHP in human blood samples and demonstrated that the concentrations of MEHP and DEHP in serum of healthy volunteers were <5 and <25 ng/ml, respectively [19]. Our results confirm their findings.

The direct injection methods using a column switching LC/MS system [19] and a solid-phase microextraction/HPLC [20] were effective in minimizing the contaminations of MEHP and DEHP during experimental procedures. However, these methods would have the potential for loading matrices into the LC/MS system or HPLC, which interfere with the accurate determination of analytes. The reliability of experimental data supported by MS/MS is one of the advantages of this method. Especially in the case of shortened cleanup steps, this advantage would be important. In our procedure, a large part of the contamination came from the solvents. Adopting the column switching system instead of the extraction steps in our procedures to decrease solvent usage would be possibly minimize the contamination and set the LOQ lower.

EU and IARC estimated that the human daily oral DEHP intake would be in the range of 5–21 μg/kg per day [21,22]. However, the concentrations of MEHP and DEHP in the serum of human that were orally administrated DEHP in

that range, have not yet been determined. After oral administration of DEHP, the concentration of DEHP in serum is lower than that of MEHP since a large part of orally administrated DEHP is absorbed after hydrolyzing to MEHP in the intestine [23,24]. The ratio of the concentration of MEHP and that of DEHP (MEHP/DEHP) in serum was 6–12:1 in rats [23,24]. In this study, MEHP/DEHP in human serum was calculated to be 2.0–4.7:1. There is difference in DEHP hydrolysis activities to MEHP among several species [12]. The DEHP hydrolysis activity of human intestine was conceived to be lower than that of the rat [12]. Assuming that the DEHP hydrolysis activity in the intestine reflect the MEHP/DEHP in serum, the large part of MEHP and DEHP detected in the serum should be sourced from the volunteers' nutrition. The concentrations of MEHP and DEHP in volunteers' nutrition did not determined in this study. There are few studies of human about relationship between the dose of orally administrated DEHP and the concentrations of MEHP and DEHP in serum [25]. To assess the daily exposure level of DEHP, determination of the concentrations of MEHP and DEHP in human serum would be informative.

The leaching of DEHP from medical devices into solutions was affected by the lipid content, the flow rate of solutions [26,27], and the concentration of the surface-active agent [28]. The exposure of DEHP to infants via TPN was estimated to be non-negligible from model studies [27]. To minimize the exposure of MEHP and DEHP to patients, improvement of medical devices using PVC, and determination of the checkpoints for handling of the medical devices would be important. Furthermore, model studies of the leaching of DEHP from medical devices, as well as investigations of the relationship between contamination and storage conditions of materials;, such as time, temperature and light would be informative to improve the medical devices. Changing DEHP in the medical device to an alternative would be effective in decreasing the exposure of MEHP and DEHP to patients. As a candidate of an alternative plasticizer for DEHP, trioctyltrimellitate (TOTM) is being used in medical devices for its minimal leaching and low hepatic toxicity [29–31]. For the safety of patients, more knowledge of the toxicities and application of TOTM in medical devices will be required. Thus, the risk assessment of medical usage of DEHP and the improvement of medical devices using DEHP should be continued. To achieve these goals, reliable methods for the measurement of MEHP and DEHP in blood is required. The method reported here would be applicable towards this end.

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Development of a simple method for predicting the levels of di(2-ethylhexyl) phthalate migrated from PVC medical devices into pharmaceutical solutions

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Abstract

This study deals with the development of a simple method for predicting the elution levels of di-2-ethylhexyl phthalate (DEHP) from medical devices made of polyvinyl chloride (PVC) by using the physicochemical properties of pharmaceutical injections as a marker. GC-MS analysis showed that the release of DEHP from medical grade PVC product was concentration-dependently increased by extraction with two kinds of lipophilic injections (Sandimmun® and Prograf®) and three kinds of surfactants (HCO-60, Tween® 80, and SDS). The solubility of lipophilic pigments such as Sudan III, methyl yellow, and 1,4-diamino-anthraquinone against these solutions were also increased in a concentration-dependent manner, in which methyl yellow showed the highest response regarding the increase of optical density (O.D.). Further, electrical conductivity and static contact angle to the PVC sheet of the solutions were also increased or decreased in the same manner. As a result of the comparative study, significant correlation was found between DEHP release levels and these three physicochemical properties, particularly methyl yellow solubility, of the solutions tested. To evaluate the relationship in detail, DEHP release levels from PVC tubing and methyl yellow solubility of 53 injections used in gynecologic and obstetric fields were determined. None of the hydrophilic medicines showed any significant release of DEHP, and all showed low solubility of methyl yellow. On the other hand, the lipophilic medicines releasing a large amount of DEHP showed high solubility of methyl yellow (greater than O.D. 0.8). These

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results indicate that a significant proportional relationship exists between DEHP release potency and methyl yellow solubility of pharmaceutical solutions, and the risk of DEHP exposure to the patients administered pharmaceuticals through transfusion set could be easily predicted by the solubility test without complicated elution tests of DEHP using GC-MS or LC-MS.

Keywords: DEHP; PVC; Medical device; Prediction; Risk assessment

1. Introduction

Phthalate esters, and DEHP in particular, have been extensively used as plasticizers due to the increased flexibility of PVC a plastic polymer used in a wide array of products including medical devices such as tubings, intravenous bags, blood containers, and catheters. DEHP is easily eluted from PVC products into not only foods but also pharmaceuticals and body fluids that come in contact with the plastic, and the migrated DEHP is directly and/or indirectly introduced into the human body (Allwod, 1986; Loff et al., 2000; Tickner et al., 2001). Some phthalates including DEHP are considered to be a toxic compound exhibiting effects similar to those of endocrine disruptors in rodents; they have antiandrogenic effects in male rats during the development of the male reproductive system and the production of normal sperm (Poon et al., 1997; Lamb et al., 1987; Tyl et al., 1988), and decrease the 17β-estradiol level in blood in female rats (Davis et al., 1994). General toxicity of DEHP has been well evaluated, and so far the result of risk assessment to human health indicates that this compound is relatively safe to humans. However, because the reproductive and developmental toxicity of DEHP to the human body is not well understood, it has recently been suggested that precautions be taken to limit the exposure of humans, particularly that of high risk patient groups such as male neonates, male fetuses, and peripubertal males, to DEHP. The concern is that DEHP's potency might have adverse effects on humans similar to those demonstrated on young rodents.

Taking the above into consideration, several agencies and official organizations in the world individually evaluated the safety of DEHP released from PVC products (Center for Devices and Radiological Health, 2001; Health Canada, 2002), and the Japanese Ministry of Health, Labor and Welfare (JMHLW) restricted the oral tolerable daily intake (TDI) value to 40–140 μg/kg/day.

It is very important that the exposure amount be exactly determined to conduct a risk assessment of the effect of DEHP on human health. Although some studies on the elution of DEHP from PVC medical devices have been performed as one of the JMHLW projects (Haishima et al., 2004; Inoue et al., 2003a,b; Takatori et al., 2004), it is not easy to identify the release behavior of DEHP from the variety of PVC products used in Japan by elution test under conditions that are the same as or similar to those of medical use. In addition, analytical methods having high sensitivity, precision, selectivity of quantitative ions, and low background, such as tandem LC-MS, high resolution GC-MS, and column-switching LC-MS methods, are required to determine DEHP for clinical assessment. Thus, regardless whether an investigation is in vivo or in vitro, the release test of DEHP is at present time-consuming and labor-intensive.

Jenke (2001) reported that the chemical compatibility assessment considers two distinct yet complementary mechanisms by which a device and its contacted solution can interact. These mechanisms include the migration of a chemical component out of the device and into the contacted solution (leaching) and the sorption of contained solution components by the device (binding). Alternatively, the product/device interaction can be modeled based on a rigorous scientific assessment of the physicochemical processes. Such models are based on the linear correlation of polymer/solution interaction constants with solvent/water partition coefficients (Nasim et al., 1972; Pitt et al., 1988; Hayward et al., 1990; Kenley and Jenke, 1990; Jenke, 1991; Jenke et al., 1991; Atkinson and Duffull, 1991; Roberts et al., 1991; Jenke et al., 1992). In addition, it is known that extraction occurs either by leaching or after an extracting material such as blood and pharmaceutical solutions diffuses into the PVC matrix and dissolves the plasticizer, which is relatively lipophilic. In consideration of these issues, we suspected that the release behavior of DEHP from PVC medical devices may be predicted from the physicochemical properties of

pharmaceutical injections applied to the devices, without a complicated elution test.

In the present study, to develop a simple method for predicting the release level of DEHP from PVC medical devices, we examined the relationship between the release potency of DEHP from PVC product and physicochemical properties such as the solubility of lipophilic pigments, electrical conductivity, and the static contact angle to PVC sheet, using two kinds of lipophilic injections and three kinds of surfactants as test solutions. Further, to evaluate the relationship in detail, DEHP release levels from PVC tubing and the physicochemical properties of 53 injections used in gynecologic and obstetric fields were determined.

2. Materials and methods

2.1. Chemicals and utensils

Medical grade PVC sheet for blood container and PVC tubing for transfusion set were provided by Terumo Co. (Tokyo, Japan).

Sandimmun® (50 mg/ml cyclosporine) and Prograf® (5 mg/ml tacrolimus) were provided by Novartis Pharma K.K. (Tokyo, Japan) and Fujisawa Pharmaceutical Co., Ltd. (Tokyo, Japan). The other 51 injections listed in Table 1 were purchased from commercial companies. Polyoxyethene hydrogenated castor oil 60 (HCO-60) provided by Nikko Chemicals Co. (Tokyo, Japan), polysorbate 80 (Tween® 80, ICN Biomedicals Inc., Ohio, USA), and sodium lauryl sulfate (SDS, Sigma Aldrich Japan, Tokyo, Japan) were used as surfactants. In these materials, Sandimmun®, Prograf®, HCO-60, Tween® 80, and SDS were used as pretest solutions for evaluating the relationship between release potency of DEHP and physicochemical properties of pharmaceuticals.

Methyl yellow (Wako Pure Chemical Industries, Ltd., Osaka, Japan), Sudan III (Sigma Aldrich Japan, Tokyo, Japan), and 1,4-diamino-anthraquinone (Tokyo Kasei Co., Tokyo, Japan) were used as lipophilic pigments. DEHP and DEHP-d_4 were purchased from Kanto Chemical Co. (Tokyo, Japan). Hexane, anhydrous sodium sulfate, sodium chloride of phthalate esters of analytical grade, diethyl ether of dioxin of analytical grade, and distilled water of HPLC grade were used in this study.

All utensils were made of glass, metal, or teflon, and were heated at 250 °C for more than 16 h before use.

2.2. Classification of pharmaceuticals

As shown in Table 1, based on the properties of principal drugs and additives contained in each pharmaceutical, 53 injections used in this study were divided into five groups. Expression rule on solubility of the drugs has been established in general notices in the Japanese Pharmacopoeia IX edition regarding the relationship between descriptive term and the degree of dissolution. Pharmaceuticals such as Sandimmun® and Prograf® containing principal drugs that are expressed as practically insoluble or insoluble to water in the instruction manuals were assigned to group 1 as lipophilic injections. Most of pharmaceuticals in this group were contained various additives such as surfactants, oils, glycerin, ethanol, benzyl alcohol, and so on. The principal drugs of pharmaceuticals classified into group 2 are also insoluble or very slightly soluble to water, but these drugs can be dissolved in acidic or basic solutions. Gaster®, Droleptan®, Elaspol®, Aleviatin®, Methotrexate® Parenteral, Serenace®, and Bosmin® were assigned to this group, and pH of each pharmaceutical is expressed in the instruction manuals as 4.7–5.7, 2.5–4.5, 7.5–8.5, approximately 12, 7.0–9.0, 3.5–4.2, and 2.3–5.0, respectively. Pharmaceuticals consisted of drugs that are slightly soluble or sparingly soluble to water were classified into group 3. Solubility of principal drugs contained in the pharmaceuticals assigned to groups 4 and 5 was expressed as very soluble, freely soluble, or soluble to water in each instruction manual. Pharmaceuticals of group 5 are hydrophilic injections as negative control regarding DEHP migration. Although pharmaceuticals assigned to group 4 are also hydrophilic injections, these pharmaceuticals were suspected to induce DEHP migration, because some of them are human serum products or containing chlorobutanol, phenol, and benzyl alcohol as additives.

2.3. Solubility test of lipophilic pigments

One millilitre of each surfactant solution and pharmaceutical injection was added to each lipophilic pigment (5 mg) followed by sonication for 10 min at room temperature and centrifugation at 3000 rpm for 10 min. The supernatant was passed through a membrane filter (pore size 0.2 μm) and the filtrate (100 μl) was