

# In vitro comparison of different gall stone dissolution solvents

H Vergunst, O T Terpstra, H G T Nijs, G Steen

## Abstract

**Extracorporeal shockwave lithotripsy (ESWL) of gall bladder stones leaves residual fragments that need to be dissolved by chemical solvents. In this study we compared the in vitro dissolving capacity of methyl tert-butyl ether (MTBE), mono-octanoïn, limonene, and limonene/mono-octanoïn (70%/30%). From nine sets of five human gall stones obtained at cholecystectomy, four stones were used for dissolution and the fifth was used for chemical analysis of cholesterol, calcium, and bilirubin contents. Eight sets were cholesterol stones with a mean (SD) cholesterol content of 89.9 (5.6)%. These stones dissolved completely in either solvent, often leaving sand-like debris, with the exception of one stone. MTBE dissolved cholesterol gall stones 100 times faster than mono-octanoïn and 10 times faster than limonene or the limonene/mono-octanoïn mixture ( $p < 0.001$ ). The combination of limonene and mono-octanoïn was as effective as limonene alone. Of the four solvents, MTBE is the best one to evaluate for dissolution of residual fragments after ESWL treatment of gall bladder stones.**

Extracorporeal shockwave lithotripsy (ESWL) is a promising non-surgical treatment for selected patients with cholelithiasis.<sup>1</sup> To achieve complete stone clearance, however, ESWL must be combined with adjuvant oral cholelitholysis by chenodeoxycholic or ursodeoxycholic acid, or both. Notwithstanding its simplicity and harmlessness, oral dissolution treatment is expensive and must be continued for at least six to 12 months after primary therapy by ESWL.<sup>1</sup> The use of dissolution solvents for direct percutaneous gall bladder perfusion may therefore afford a favourable alternative and is of renewed interest.

Mono-octanoïn was found to be a useful substance for direct dissolution of retained cholesterol bile duct stones by either T tube infusion<sup>2-6</sup> or by percutaneous transhepatic catheterisation.<sup>3</sup> It is also used through an endoscopically positioned nasobiliary catheter.<sup>7</sup>

Rapid dissolution of cholesterol gall stones with methyl tert-butyl ether (MTBE) has been described recently.<sup>8</sup> MTBE is structurally related to diethylether but unlike diethylether, which vaporises at body temperature, it remains liquid because of its higher boiling point (55.2°C).<sup>9</sup> It can be delivered to the gall bladder or the common bile duct by a nasobiliary or a percutaneous transhepatic catheter.<sup>8 10-14</sup>

The monoterpene, limonene, is obtained from the peel of citrus fruits. It seemed to be an excellent dissolution solvent in vitro on human

cholesterol gall stones.<sup>15</sup> Complete dissolution of duct stones was reported by Igimi *et al* in 13 of 15 patients using a 97% d-limonene solution.<sup>16</sup> More recently, the authors changed the prescription of the d-limonene preparation to 70% d-limonene and 30% middle chain monoglyceride to improve efficacy and reduce the side effects of the previous preparation.<sup>17</sup> In vitro studies showed extraordinary synergism when d-limonene and mono-octanoïn were used in a 3:2 mixture to dissolve cholesterol stones.<sup>15</sup>

In this study we compared the gall stone dissolving capacity of four different dissolution solvents in vitro: MTBE, mono-octanoïn, a 97% limonene preparation and a limonene/mono-octanoïn mixture.

## Methods

### STONES

Nine sets of five gall stones were obtained from nine consecutive patients who underwent elective cholecystectomy for symptomatic gall bladder stones. By gross observation, the stones comprising one set were comparable in morphology and size, and assumed to be of similar chemical composition. The stones were stored at 4°C in phosphate buffered saline (PBS), containing 50 000 IU/l penicillin and 50 000 µg/l streptomycin. One stone from each set was used for chemical analysis of cholesterol, calcium, and bilirubin contents, as described previously.<sup>18</sup> The remaining four stones were used to study dissolution by different solvents.

The 36 stones used for dissolution were placed on blotting paper and allowed to dry in air for one hour before weighing and measuring the maximum diameter of each stone. A Mettler PK 300 digital balance (Mettler Instrumente AG, Greifensee, Switzerland) was used for weighing.

### DISSOLUTION SOLVENTS

The following dissolving agents were used: mono-octanoïn (glyceryl-1-mono-octanoate, Capmul 8210, from Stokely USA Inc, Oconomowoc, Wisc, USA); methyl tert-butyl ether (MTBE, from Merck-Schuchardt, Schuchardt, Germany); d-limonene (d-p-mentha-1,8-diene), Tween 80 (polyoxyethylene-sorbitan-mono-oleate), and Span 80 (sorbitan mono-oleate) (from Sigma Chemical Company, St Louis, MO, USA).

The 97% limonene preparation was prepared by adding 2.1 parts of Tween 80 and 0.9 parts of Span 80 to 97 parts of d-limonene. The limonene/mono-octanoïn mixture was prepared by adding 70 parts of d-limonene to 30 parts of mono-octanoïn.

Departments of Surgery and Clinical Chemistry, University Hospital Dijkzigt and Erasmus University Rotterdam, Rotterdam, The Netherlands  
H Vergunst  
O T Terpstra  
H G T Nijs  
G Steen

Correspondence to:  
Dr O T Terpstra, Professor of Surgery, University Hospital Dijkzigt 3015 GD Rotterdam, The Netherlands.

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TABLE I Percentages of dry weight of cholesterol, calcium, and bilirubin in nine different sets of gall stones

Set no	Cholesterol (%)	Calcium (%)	Bilirubin (%)
1	92.3	0.8	0.5
2	91.4	0.0	0.7
3	92.8	0.7	0.1
4	86.3	2.0	0.1
5	96.9	0.0	0.1
6	0.0	*	10.0
7	91.3	0.0	0.1
8	78.1	*	0.4
9	90.2	1.3	0.2

\*Amount of material insufficient for chemical analysis. One stone from each set was used for chemical analysis.

#### DISSOLUTION EXPERIMENT

The stones were immersed individually in test tubes containing 10 ml of one of the four different solvents. The tubes were sealed off, placed in a constant temperature (37°C) water-bath, and gently agitated. For reweighing, the stones were lifted out of the solutions using a spatula or by filtration in the case of multiple residues. Stones were blotted dry before weighing every 10 minutes up to two hours; hourly up to six hours; daily from 24–72 hours, and then at three to four day intervals up to 14 days. At the same intervals aliquots (100 µl) of the dissolution solvents were taken for determination of the cholesterol content by the CHOD PAP method.<sup>19</sup> After weighing, the stones or residues

were returned to the original test tubes. The different solvents were not replaced during the study. Dissolution was defined as a reduction in stone mass, the residual debris not exceeding 2 mm in diameter.

#### DATA ANALYSIS

Stone weights at set times were expressed as percentages of the initial stone weight. The relative reduction in stone weight over time was related to the relative recovery of cholesterol from the medium. The 25% and 50% dissolving times were calculated by solving a four parameter logistic equation<sup>20</sup> using ALLFIT 2.7 (software kindly provided by Dr D Rodbard, NIH, Bethesda, MD, USA). Data were compared using non-parametric analysis of variance (Friedman's test) and the Mann-Whitney test.

#### Results

Table I gives the results of chemical analyses of a single stone from each set. The stones comprising set 6 were black in colour and had an irregular surface – typical criteria for pigment stones. These stones consisted of 10% bilirubin and did not contain any cholesterol. The remaining sets were smooth surfaced, rounded or faceted, and for the greater part yellowish stones, largely composed of cholesterol. The mean (SD) percentage of cholesterol in the latter stones was 89.9 (5.6)%.

In each of the four solvents, the pigment stones (set 6) disintegrated partly or completely into multiple fragments. The weights of these stones were reduced by 20–35% within the first two to six hours; thereafter a further decrease in weight did not occur (Fig 1).

The remaining cholesterol stones incubated in MTBE, mono-octanoin, limonene, and limonene/mono-octanoin had mean (range) weights of 845 (110–1634), 702 (132–1563), 787 (123–1854), and 787 (79–2046) mg respectively. The mean (range) diameters were 13.7 (6.5–20.5), 12.7 (6.8–19.1), 13.7 (6.8–21.0), and 12.8 (6.0–18.1) mm respectively. For the four different solvents, the weights and diameters of the stones did not differ significantly.

During dissolution of the cholesterol stones in MTBE, limonene, and limonene/mono-octanoin most stones broke into multiple fragments; in mono-octanoin this occurred in two cases only. All cholesterol stones dissolved completely in either solvent, except one stone from set 9, which was reduced by only 50%. The stones from set 5, which had the highest percentage of cholesterol (Table I), dissolved completely without leaving any residue. Dissolution of the other stones resulted in a sand-like debris (except for stones from set 9).

Figure 2 shows the results for the eight sets of cholesterol stones for each time point; data are expressed as the mean percentage of the initial stone weight. In MTBE, stones dissolved within two hours. In mono-octanoin, limonene, and limonene/mono-octanoin, however, ±85%, ±40%, and ±20% respectively were still left after 24 hours. Dissolution with mono-octanoin was completed after 10 days.

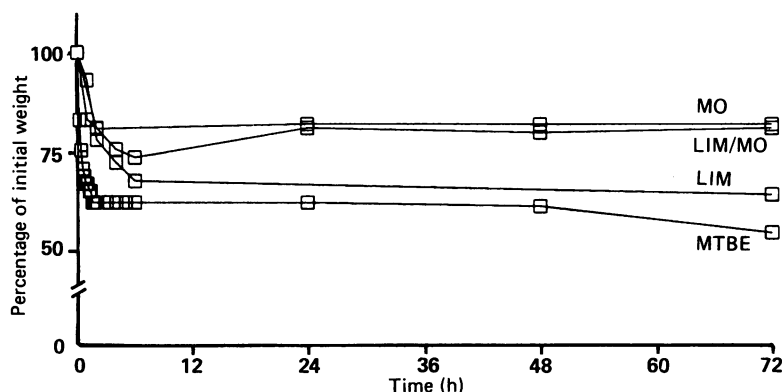


Figure 1: Relative reduction in weight of pigment stones (set 6) achieved by methyl tert-butyl ether (MTBE), mono-octanoin (MO), limonene (LIM), and limonene/mono-octanoin (LIM/MO).

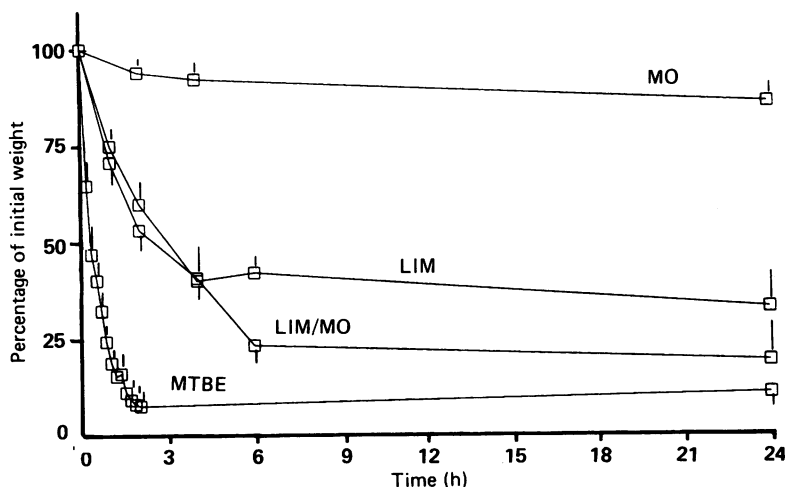


Figure 2: Relative reduction in weight of eight sets of cholesterol stones in four different solvents, expressed as the percentage of initial stone weight for methyl tert-butyl ether (MTBE), mono-octanoin (MO), limonene (LIM), and limonene/mono-octanoin (LIM/MO). Data are mean (SEM) values.

TABLE II Dissolving times of methyl tert-butyl ether (MTBE), mono-octanoin (MO), limonene (LIM), and limonene/mono-octanoin (LIM/MO) at which the initial weights of eight cholesterol stones were reduced by 25% and 50%. Values represent medians with 25th–75th centiles in parentheses

Solvent	Dissolving time (h) to achieve weight reduction of	
	25%	50%
MTBE	0.2 (0.1–0.2)	0.3 (0.2–0.5)
MO	21.1 (13.4–25.2)	45.5 (34.8–61.8)
LIM	1.2 (0.7–2.2)	3.4 (1.7–5.1)
LIM/MO	1.0 (0.6–2.2)	2.1 (1.3–4.0)

MTBE v MO, LIM, and LIM/MO:  $p < 0.001$ ; MO v LIM, and LIM/MO:  $p < 0.001$ ; LIM v LIM/MO: NS.

In Table II the median 25% and 50% dissolving times are given. MTBE dissolved cholesterol gall stones 100 times faster than mono-octanoin ( $p < 0.001$ ) and 10 times faster than limonene or limonene/mono-octanoin ( $p < 0.001$ ). The combination of limonene and mono-octanoin was as effective as limonene alone.

The four parameter logistic equation proved to be an adequate mathematical description for the weight reduction of the stones in either solvent. A typical example is shown in Figure 3.

Figure 4 illustrates the relative reduction in stone weight and the relative recovery of cholesterol from mono-octanoin, limonene, and limonene/mono-octanoin, expressed as a percentage of the initial stone weight. Data on MTBE are not available due to technical difficulties caused by the volatility of the solvent. The reduction in stone weight paralleled the recovery of cholesterol, showing that the reduction in stone weight was due to dissolution of cholesterol.

**Discussion**

The combination of chenodeoxycholic and ursodeoxycholic acid has been shown to be effective and safe in dissolving cholesterol stones in selected patients.<sup>21,22</sup> However, the slow process of stone dissolution,<sup>15,23</sup> the high recurrence rate after stopping treatment,<sup>24</sup> and the low percentage of patients that fulfil the selection criteria<sup>25</sup> are considerable disadvantages of oral dissolution therapy.

Recently, oral cholelitholysis has become more important as adjuvant treatment after ESWL of gall bladder stones for dissolution of

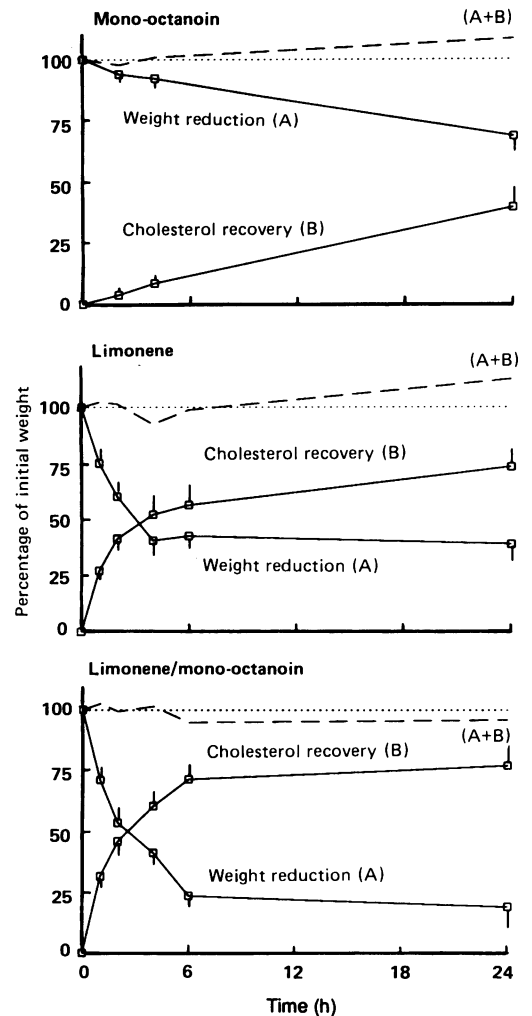
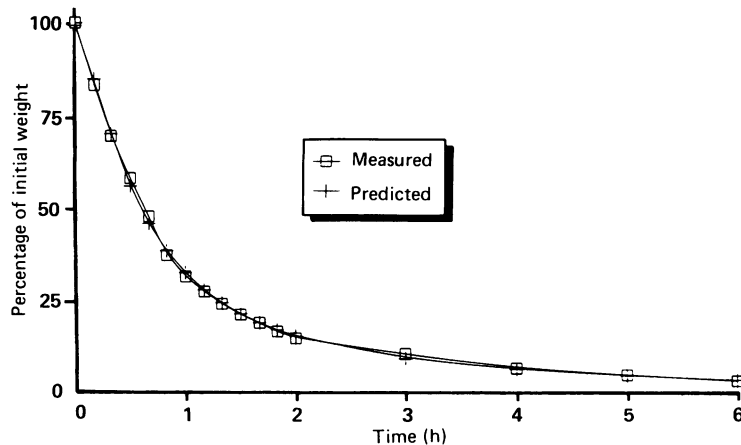


Figure 4: Relative reduction in stone weight and relative recovery of cholesterol from medium, expressed as percentage of initial stone weight for mono-octanoin, limonene, and limonene/mono-octanoin. Data are mean (SEM) values.

Figure 3: Relative reduction in stone weight in methyl tert-butyl ether (MTBE) (set 8), compared with the predicted reduction in stone weight by the four parameter logistic equation.



the residual fragments.<sup>1,26</sup> Dissolution therapy with bile desaturating agents is enhanced considerably by preceding stone fragmentation.<sup>27</sup> Nevertheless, the expensive adjuvant oral dissolution treatment is still lengthy. This may reduce the cost effectiveness of combined ESWL and chemolytic treatment as an alternative for cholecystectomy. Therefore, notwithstanding its more invasive nature, percutaneous dissolution treatment might be optional for dissolving the fragments remaining after ESWL.<sup>28–31</sup> For patients with large or numerous stones, who are currently excluded from ESWL treatment, contact dissolution may broaden the applicability of ESWL.<sup>1</sup>

In a model dissolution system, it seemed that the initial stone diameter and matrix content determined the dissolution rate rather than the cholesterol content.<sup>32</sup> Fragmentation of gall stones increases the surface for solvent-crystal contact thereby accelerating stone dissolution. Furthermore, disruption of concentric rings of matrix within stones may also contribute to the ease of dissolution. Laser fragmented gall stones dissolved in vitro significantly faster in MTBE than did intact stones.<sup>33,34</sup> Calcified stones cannot be dissolved by MTBE. Nevertheless, when calcium is distributed in an outer shell or inner core, mechanical fragmentation increases the

efficacy of MTBE dissolution.<sup>28,34</sup> We found a significant acceleration in gall stone dissolution by MTBE after ESWL in a pig model, irrespective of stone calcification (Vergunst *et al*, unpublished observations).

In this study we compared four different solvents possibly suited for post ESWL contact dissolution. The cholesterol dissolving capacity of different limonene preparations *versus* MTBE and mono-octanoin, respectively, had not previously been compared in vitro.

We found that MTBE dissolved cholesterol stones in vitro more than 100 times faster than mono-octanoin. This finding corroborates the previously reported superior dissolving capacity of MTBE compared with mono-octanoin, even for stones with a cholesterol content of only 40%.<sup>35</sup> Compared with the limonene preparations MTBE dissolved comparable stones 10 times faster. It has been reported that pigment stones are not dissolved by MTBE.<sup>35,36</sup> The pigment stones in our study, however, were partly dissolved by either solvent. Since these stones did not contain any cholesterol, the weight reduction must be caused by dissolution of other organic constituents.

In conclusion, MTBE, which has been used clinically for dissolution of gall bladder stones without severe side effects or complications,<sup>8</sup> is the most potent cholesterol solvent now available. Combining ESWL and contact dissolution therapy probably renders more patients with cholecystolithiasis eligible for non-surgical treatment. Of the 4 solvents, MTBE is the best one to evaluate for dissolution of residual fragments after ESWL.

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