A New Dissolution Effect of DMO on Human Pancreatic Stone —— In vitro Study ——

Kazunari HINO, Keiko TAKATORI, Ryosuke YAMAMOTO, Seiji IDEGUCHI, Kenji OHMOTO, Tsuneyo OHUMI, Shinichiro YAMAMOTO and Yutaka HIRANO

Division of Gastroenterology I, Department of Medicine, Kawasaki Medical School, Kurashiki 701-01, Japan

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ABSTRACT. In this study, we examined the dissolution effect of dimethadione (DMO) on pancreatic stones, when kept at 37℃ in a DMO 0.05 M $NaHCO_3$ saline solution which was replaced once a week, were partially dissolved during a 12-week period. The decreasing stone weight ratios were 38% (1.0 g/l DMO), 41% (0.5 g/l), 7% (0.1 g/l), and 2% (control). The DMO solution induced a concentration-dependent increase in the solubility of the pancreatic stones associated with a concentration-dependent fall in the solution pH. The eluted calcium concentration in the solution was measured after one week's incubation, and then the decrease in stone weight in theory was calculated. The decrease in stone weight in practice, however, was more than the calculated weight in theory. To determine the reason for this discrepancy, we examined the solution microscopically for sediment and found amorphous substances indicating a concentration-dependent increase in the amount of sediment. These substances resembled artificially broken pancreatic stones and they were dissolved by bubbles following the addition of acetic acid. This phenomenon suggests that these substances and pancreatic stones consist of $CaCO_3$. Therefore, it appears DMO has the potency not only to dissolve $CaCO_3$, but also to break pancreatic stones into small pieces.

Key words : pancreatic stone dissolution therapy — dimethadione(DMO) — calcium carbonate

Chronic pancreatitis is an irreversible disease characterized by pancreatic stones in the pancreatic duct or parenchyma, for which there has been no effective treatment until recently. However, recently, dissolution therapy for pancreatic stones in man and dogs by the oral administhation of trimethadione (TMO) was reported by Noda *et al.*[1,2]

TMO (3,5,5 - trimethyl - 2,4 - oxazolidinedione) is used as an antiepileptic agent (MINO-ALEVIATIN®) and is a precursor of DMO (5,5-dimethyl-2,4-oxazolidinedione, Dimethadione)(Fig. 1). The in vitro experiments of Noda *et al.* indicated that DMO induces a concentration-dependent increase in the solubility of calcium carbonate ($CaCO_3$), which is the main constituent of pancreatic stones. In addition, it was also found that human and dog pancreata are capable of excreting a weak organic acid (DMO) depending on the concentration of DMO in plasma.[1]

日野一成，高取敬子，山本亮輔，井手口清治，大元謙治，大海庸世，山本晋一郎，平野　寛

Sahel and Sarles[3] once reported that oral citrate therapy was effective for the dissolution of pancreatic stones. With regard to that report, Noda *et al.* mentioned that DMO has almost the same potency as tartaric acid and citric acid.[1]

Although when a pancreatic stone dissolves, Ca and CO_3 dissolve together, considered previous reports regarding the solubility of $CaCO_3$ only the decrease in Ca and did not consider CO_3. It seems, however, that the existance of CO_3 must not be ignored because the molecular weight of CO_3 in $CaCO_3$ is sizable; i.e., molecular weight of $CaCO_3$ is about 100 and that of CO_3 is about 60. Therefore, in this report we studied the mechanism of decreasing stone weight with regard to not only the amount of Ca but also the amount of CO_3.

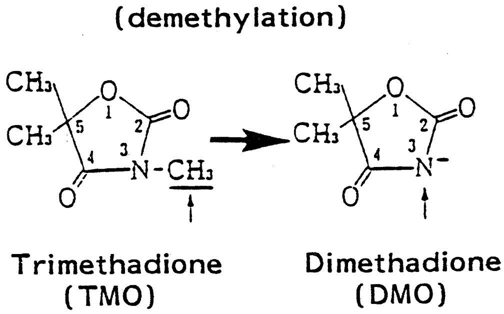

Fig. 1. TMO is converted into DMO by metabolic N-demethylation.

MATERIALS AND METHODS

1. Pancreatic stones (Fig. 2)

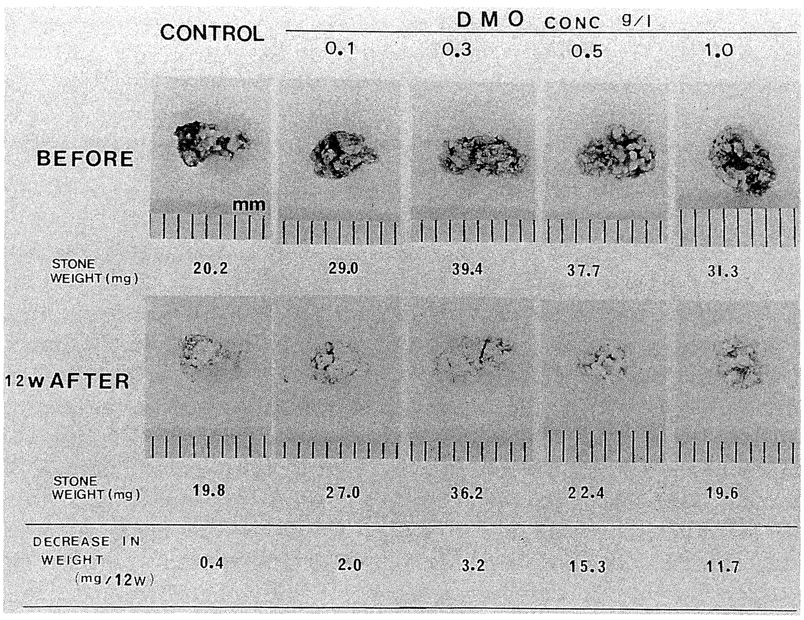

Fig. 2. Morphological change in pancreatic stones and in stone weight in various DMO solutions during a 12 week period.

Pancreatic stones were obtained at autopsy from a 35-year-old Japanese male with severe alcoholic chronic pancreatitis. The principal composition of about 100% of the pancreatic stones as determined by infrared absorption was calcium carbonate.

2. DMO solution

Various amounts of DMO were dissolved in a 0.05 M $NaHCO_3$ saline solution as a solvent. The DMO concentrations were 0 g/l as the control, and 0.1 g/l, 0.3 g/l, 0.5 g/l and 1.0 g/l.

3. Procedures

Pancreatic stones were kept at 37℃ in a shaking state in 20 ml of DMO-0.05 M $NaHCO_3$ saline solution, which was replaced with fresh solution once a week. After each one week incubation period, the calcium concentration in each DMO solution was measured six times with an atomic absorption spectrophotometer (HITACHI 180-160). The pH was measured 10 times and the dry stone weight was measured 12 times. The sediments in each solution were also examined microscopically after incubation. Sediments were obtained by centrifugation at 1700 g for 30 min.

RESULTS

1. Decreases in stone weight

Fig. 2 shows morphological changes in pancreatic stones and change in stone weight in various DMO solutions during a 12 week period. There was a clear reduction in the size and weight of stones in the higher concentrations of DMO solution (0.5 g/l, 1.0 g/l). They also became polyporus in shape. The stones in the lower concentrations of DMO solution (0.1 g/l, 0.3 g/l) also showed similar but milder changes and the stone in the control solution showed slighter change.

2. Decreasing ratios in stone weight

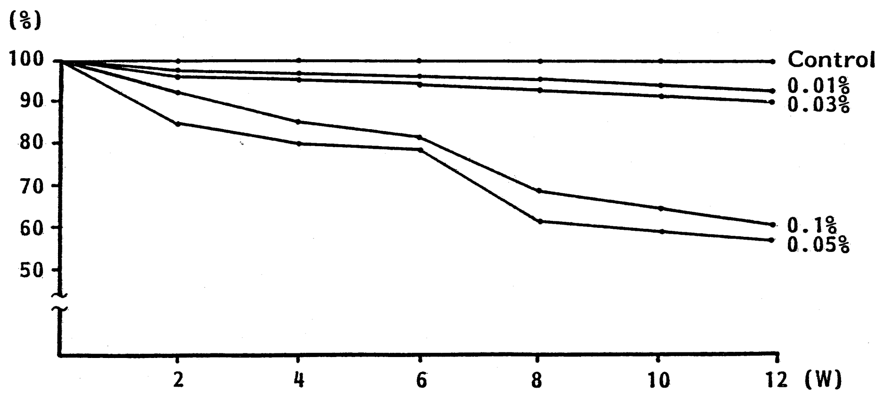

Fig. 3. Decreasing ratio in stone weight.

As illustrated in Fig. 3, the decreasing ratios in stone weight were 41% (0.5 g/l), 38% (1.0 g/l), 8% (0.3 g/l), 7% (0.1 g/l), 2% (control). The decreasing ratio was clearly larger in the higher concentrations of DMO solution than in the lower ones.

3. Change in solution pH

Table 1 indicates that higher concentrations of DMO solution had lower pH values immediately after preparation. These rose after one week incubation in every concentration of DMO solution.

TABLE 1. Change in pH of solutions.

	Before	After one week incubation
control	8.3±0.08	8.5±0.09
0.1 g/l	7.8±0.42	8.5±0.04
0.3 g/l	7.8±0.16	8.5±0.03
0.5 g/l	7.7±0.29	8.3±0.05
1.0 g/l	7.5±0.35	8.4±0.18

(n=10 times)

4. Concentrations of calcium eluted into solution during one week incubation

Calcium was not detected in just prepared solutions but it was eluted during one week incubation with increase in the DMO concentration. The calcium concentrations in each solution during one week are shown in Table 2A. In the control solution, only a small amount of calcium was eluted. The decrease in calcium during a 12 week period in theory should have been no more than 2.0 mg, as shown in Table 2B. Considering the existence of CO_3

TABLE 2. Calcium and $CaCO_3$ concentration.

DMO conc g/l	A mg/l/week	B mg/12week	C mg/12week	D mg/12week
control	0.03±0.01	0.01	0.025	0.4
0.1	2.5±1.2	0.90	2.25	2.0
0.3	3.0±1.5	1.08	2.70	3.2
0.5	4.0±2.0	1.44	3.60	15.3
1.0	4.5±3.0	1.80	4.50	11.7

A : Mean±SD of calcium concentrations eluted into solution during one week incubation. (n=6 times)
B : Maximum decrease in calcium weight during a 12 week period in theory.
C : Maximum decrease in $CaCO_3$ weight during a 12 week period in theory.
D : Decreased pancreatic stone weight in practice.

as a component of pancreatic stones, the maximum decrease in $CaCO_3$ during a 12 week period in theory using the data of Table 2B is shown in Table 2C. Both formulas are described in the following discussion. The real decrease in pancreatic stone weight is shown in Table 2D, the exception of the solution containing 0.1 g/l of DMO, the real decrease in weight is much more than the decrease in theory (Table 2C).

5. *Microscopic findings of sediment in solution*

Amorphous substances were observed as sediment and the amount of these substances increased with the increase in DMO concentration (Fig. 4B,C,D,E). Only a small amount of these substances were observed in the control solution (Fig. 4A). The shape of the substances were similar to that of the artificially crushed human pancreatic stone shown in Fig. 4F. Fig. 5A shows sediments of the DMO solution. The sediments were not visible macroscopically (AA−) but they produced small gas bubbles when 3% acetic acid (AA+) was added. Fig. 5B shows an artificially crushed pancreatic stone. Very small, white substances were seen in the saline solution under a cover glass (AA−). These substances also produced gas bubbles in a similar manner to those in Fig. 5A (AA+) when acetic acid was added to them, and dissolved.

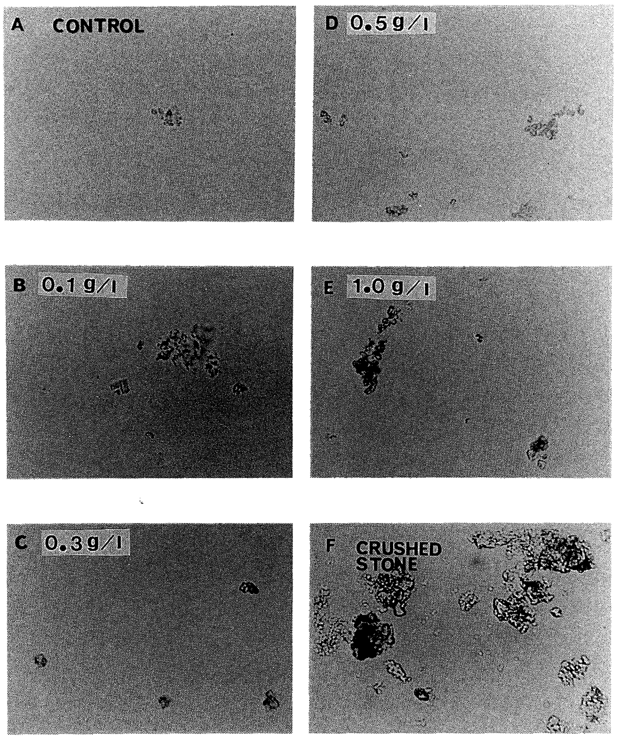

Fig. 4. Microscopic findings of sediment (× 400) in various DMO solutions are compared with those of a crushed pancreatic stone.

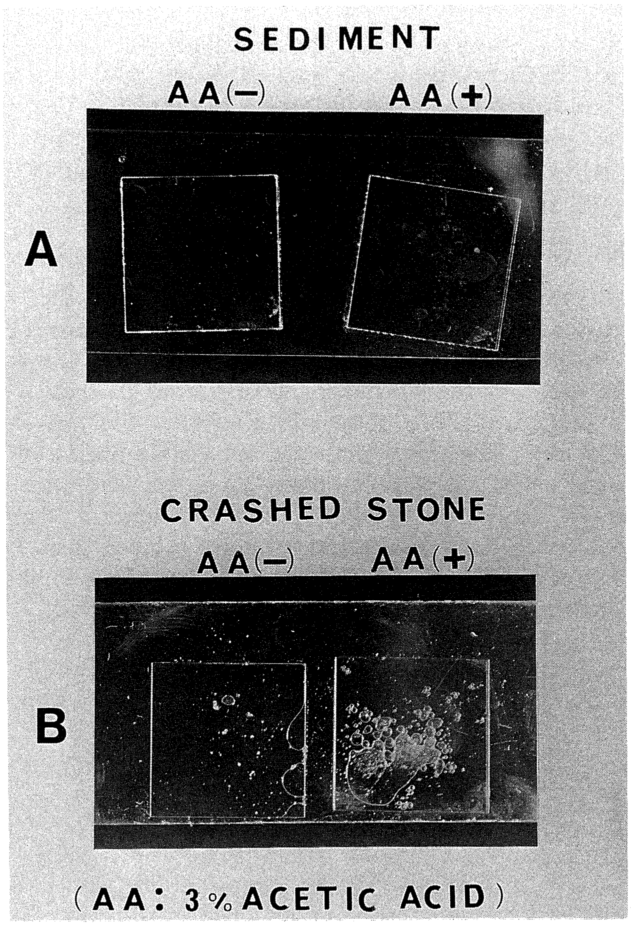

Fig. 5. Sediment of solution and the crushed pancreatic stone dissolve in acetic acid with the evolution of gas.

DISCUSSION

Based on in vitro and in vivo studies, Noda *et al.* suggested that DMO could be potentially useful for pancreatic stone dissolution.[1,2] They found that pancreatic stones, when kept at 37℃ in a DMO-0.05M $NaHCO_3$ aqueous solution which was replaced once a week, were completely dissolved in 10 g/l, 5 g/l, and 2 g/l of DMO solution in 5, 11 and 58 weeks, respectively. The stones in 1.0 g/l, and 0.5 g/l of DMO solution were partially dissolved during the observed 96 week-period, but only a 3 mg decrease in the stone weight was observed in the $NaHCO_3$ solution used as a control.[4] In addition, they showed that the maximum DMO concentration in pure human pancreatic juice was 0.15 to 0.35 g/l under a condition of oral administration for three or four consecutive days of 1.3 to 2.0 g/day of TMO as the precursor of DMO.[5] They also recommended 1.2-1.5 g of TMO as the daily intake.[2] According to their findings, therefore, it might be impossible to obtain 2.0 g/l of DMO excretion in pancreatic juice. The concentration of 2.0 g/l is the lowest value confirmed to be enough to dissolve stones completely. Therefore, we studied the reduction effect of a lower dose of DMO, under 1.0 g/l in this study. Based on the following findings we believe that we obtained better results than Noda. Stone weight decreased 41% in 0.5 g/l of DMO after only 12 weeks. These results were in marked contrast to Noda's findings of 24% in 0.5 g/l of DMO[4] after 96 weeks. Stones in DMO solution lower than 0.5 g/l (Fig. 2) showed milder change in decreasing ratios for 12 weeks. Based on the present in vitro study, we believe that DMO can reduce and possibly dissolve pancreatic stones.

To ascertain the mechanism of dissolution, we next studied the relationship between the solution pH and solubility of the stones, and measured the quantity of calcium eluted into solution after one week of incubation. With regard to pH, Noda reported a concentration-dependent increase in the solubility of $CaCO_3$ associated with a concentration-dependent fall in solution pH.[4] In our investigation, similar results were obtained (Table 1). As for the eluted calcium concentration, Noda studied the solubility of $CaCO_3$ in 0 to 1.0 g/l of DMO aqueous solution in vitro and reported the calcium concentration reached a plateau at 10-15 min in 0.1 and 1.0 g/l of DMO solution. They also showed that the plateau level of the calcium concentration was about 0.03 g/l in 0.1 g/l of DMO and 0.2 g/l in 1.0 mg/l of DMO.[4] The calcium concentrations in Noda's study and ours were compared. Generally, the concentrations in our study were larger than theirs, especially in 0.1 g/l. We believe these differences are based on the design of the experiment. At this point, our design seems more practical for study of the relationship between a decrease in stone weight and the concentration of calcium eluted into the solution. Next, we calculated the maximum decrease of calcium and $CaCO_3$ in theory using the following formula. This calculation was made on the assumption that the maximum dose of Table 2A was eluted into the solution.

The maximum decrease of calcium during the observed period (mg) = the maximum eluted calcium concentration in a week (mg/l)$\times$0.02$\times$12 (observed week period) (The coefficient 0.02 is necessary because each solution volume was 20 ml.)

The maximum decrease of $CaCO_3$ during the observed period (mg) = the maximum decrease of calcium during the observed period (mg)$\times$100/40 (100/40 indicates the molecular weight of $CaCO_3$, which is about 100, and that of Ca, which is about 40.)

Our results indicated that the decrease in stone weight in practice is more than the eluted $CaCO_3$ concentration in theory with the exception of 0.1 g/l (Table 2B,C,D). To determine the reason for this discrepancy, we examined the solution microscopically for the existence of sediments. As a result, we found amorphous substances as sediments and also noted a DMO concentration-dependent increase in a number of them. We thought the sediments might be small broken pancreatic stones. Therefore, we observed artificially broken pancreatic stones microscopically and found substances very similar to the sediments (Fig. 4). The shape of both was consistent with the textbook description of $CaCO_3$ microscopically.[6] We added acetic acid to the solution including sediment because $CaCO_3$ dissolves in acetic acid with the evolution of carbon dioxide gas.[6] Small gas bubbles were seen in the solution under a cover glass (Fig. 5A), and these were also observed when acetic acid was added to artificially crushed pancreatic stones (Fig. 5B). These findings indicate that the substances were small broken pancreatic stones and that they were composed of $CaCO_3$. These sediments might have been produced by physical energy, since during the incubation period. The pancreatic stones were kept in a shaking state. In fact, there was some sediment in the control solution. In addition, the decrease in stone weight in practice was 16 times more than the decrease in $CaCO_3$ weight in theory. This indicates the main mechanism of the decrease in stone weight in the control might be due to physical energy. In a 0.1 g/l DMO solution the decrease in $CaCO_3$ in theory is almost equal to that in practice. However elution of the maximum dose of calcium is difficult and some sediment was also observed. Therefore, in a 0.1 g/l DMO solution, it seems the process of destruction of stone might occur. In addition a DMO concentration - dependent increase in the amount of sediment was observed in solutions containing more than over 0.3 g/l DMO. Therefore, DMO might have the potency not only to dissolve $CaCO_3$, but also to break pancreatic stones into small pieces.

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