

PANCREATIC HISTOLOGY IN PATIENTS UNDER LONG TERM CORTICOSTEROID TREATMENT

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SUMMARY: In 52 autopsy cases (30 cases treated with corticosteroid and 22 cases not treated), changes of the pancreas due to corticosteroid administration were histologically studied. Light microscopy revealed a significantly more frequent appearance of PAS positive materials inside of the acinar lumen, goblet cell metaplasia and hyperplasia of the pancreatic duct epithelium and spotty necrosis of the acinar cells in corticosteroid-treated cases than in non-treated cases. The mean number of dilated acini containing PAS positive materials (DAPPM) in ten 400 power fields was 1.63 ± 2.0 in the non-treated group and 3.76 ± 2.6 in the corticosteroid-treated group ($p < 0.01$). Thus, these findings seem to play an important role in outflow disturbance of pancreatic juice. Electron microscopy revealed filamentous and electron dense materials filling in the dilated acinar lumen, probably representing pancreatic juice with high viscosity and degraded on products of exocrine pancreatic cells.

INTRODUCTION

The possibility of pancreatic dysfunction in patients under corticosteroid treatment has been emphasized repeatedly during the past 40 years since the report of Stephenson *et al.*¹⁾ in 1952 regarding a case of acute hemorrhagic pancreatitis in response to cortisone therapy. Mallory *et al.*²⁾ described various drugs implicated in pancreatic dysfunction, including corticosteroids. Since the case report by Stephenson¹⁾ in 1952, many subsequent cases confirmed the injurious effect of corticosteroids on the pancreas³⁻¹⁴⁾.

In 1981, on the other hand, Steinberg *et al.*¹⁵⁾ expressed a different opinion on the effect of corticosteroids on the pancreas. In support of his doubts on any corticosteroid effect, he pointed out the absence of studies on the effect

of other drugs clinically used in combination with corticosteroids, the much higher dose of corticosteroids used in animal experiments compared to the doses clinically used in general and lack of reproducible histopathological changes of the pancreas.

Along with progress of clinical medicine, however, the frequency and dose of corticosteroid treatment increased, and combined administration of various drugs have also been increasing. In many patients seen in clinic the serum amylase rises along with the initiation of corticosteroid administration, strongly suggesting the possibility of some influence on the pancreas. In 1956, Stumpf *et al.*¹⁶⁾ and Bencosme¹⁷⁾ reported histological findings such as acinar cell degeneration, irregularly dilated acinar spaces, retention of secretions, cell infiltration and fibrosis in the pancreas of rabbits given corticosteroids.

In the present study, the histology of the pancreas was studied in autopsy cases divided into a group treated and a group not treated with corticosteroids, with the emphasis on the observed frequency of DAPPM. In some cases, electron microscopic studies were also performed.

MATERIALS AND METHODS

Among the autopsy cases performed during the 4 years since the inauguration of Medical College of Oita, 52 cases undergoing autopsy shortly after death (within 3 hours) were studied, including 30 cases (mean age 58.3 years, ranging from 16 to 76; 19 males and 11 females) treated with corticosteroid and 22 cases (mean age 62.3 years, ranging from 39 to 81; 16 males and 6 females) untreated, without significant difference in the age and sex distribution between these two groups. (Table 1).

Following cases were excluded from this study.

- 1) Those in which hyperamylasemia was already noted on admission.
- 2) Those with definite pancreatic and bile duct diseases.
- 3) Those with pancreatic involvement by metastasis or infiltration of malignant tumor.
- 4) All cases of collagen-vascular diseases.
- 5) Cases of chronic renal failure.
- 6) Those with history of abdominal operations.
- 7) Those in which the duration of administration or total dose of the corticosteroid is unclear.

Those cases in which corticosteroids were used immediately before death as a life-saving measure in a single dose were classified as cases not treated with corticosteroid.

Light microscopic examinations were performed from the autopsy specimen using the body of the pancreas a rule. After fixation in 20% formalin, the sections were stained by hematoxylin and eosin (HE), periodic acid-Schiff (PAS), and Azan-Mallory methods.

In order to prepare electron microscopic sections, specimens were obtained from the same site as that for the light microscopic specimens. After per-fixation in 2% glutaraldehyde, the specimens were fixed in 1% osmic acid and embedded in Epon 812. The ultra thin sections

Table 1. SUBJECTS

1. Corticosteroid treated group (age: 58.3±15.9 yrs)	
leukemia	14
malignant lymphoma	6
lung cancer	4
hepatocellular carcinoma	2
liver cirrhosis	2
miscellaneous	2

	30 cases
2. Non-treated group (age: 62.3±12.9 yrs)	
hepatocellular carcinoma	9
lung cancer	3
pneumonia	3
leukemia	2
myocardial infarction	2
miscellaneous	3

	22 cases

total	52 cases

were subjected to a uranium lead double stain. Since these were autopsy cases, only the materials inside of pancreatic duct were examined.

Administered doses of corticosteroid in all patients were converted into prednisolon equivalents according to the method of Hollander¹⁸⁾.

For the statistical analysis, Student's t-test for unpaired data and χ^2 test were employed. Significance was established at $p < 0.05$.

RESULTS

1) Light microscopic findings in the group treated with corticosteroid and the group not treated with corticosteroid

Table 2 summarizes the light microscopic findings in the group treated with corticosteroid. Findings of the pancreatic duct epithelium, inside of the acinar lumen, stroma and parenchyma of the acinar cells were distinguished and the frequency of these appearance were statistically analyzed between corticosteroid treated group and non-treated group. In the pancreatic duct epithelium, goblet cell metaplasia and hyperplasia occurred with more frequency in the group treated with corticosteroid ($p < 0.05$). No significant difference was notes in the inflammatory cellular infiltration of the pancreatic duct between treated and non-treated groups, but PAS positive materials appeared

Table 2. Histopathological findings of the pancreas
— comparison between corticosteroid treated and non-treated group —

	pancreatic duct epithelium			acinar cell				
	goblet cell metaplasia	hyper plasia	inflammatory cells infiltration	inside of acinar lumen	stroma		parenchyma	
				PAS positive materials	inflammatory cells infiltration	fibrosis	spotty necrosis	fatty necrosis
corticosteroid treated group (n=30)	16/30 (53.3%)	13/30 (43.3%)	1/30 (3.3%)	25/30 (83.3%)	8/30 (26.7%)	14/30 (46.7%)	8/30 (26.7%)	2/30 (6.6%)
non-treated group (n=22)	5/22 (22.7%)	3/22 (13.6%)	1/22 (4.5%)	7/22 (31.8%)	3/22 (13.6%)	8/22 (36.4%)	1/22 (4.5%)	1/22 (4.5%)
χ^2	4.398	5.255	0.050	14.231	1.292	0.522	4.340	0.105
<i>P</i>	<0.05	<0.05	N. S.	<0.01	N. S.	N. S.	<0.05	N. S.

(n=52)

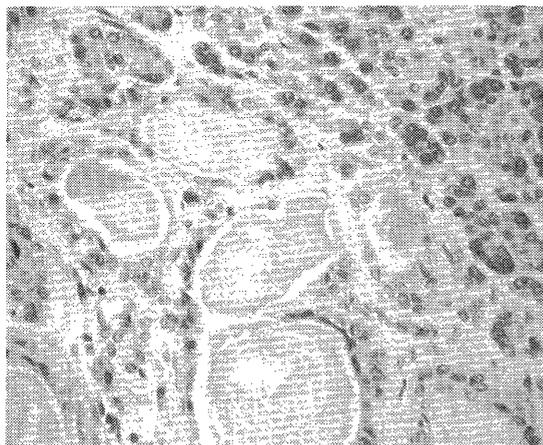


Fig. 1. A typical finding of dilated acini containing PAS positive materials (DAPPM). PAS positive materials filled the markedly dilated acinar lumen, containing lamella structures in some parts (HE, $\times 400$).

more frequently in the treated group ($p < 0.01$). Among the findings in the pancreatic acini, no significant changes such as infiltration by inflammatory cells and fibrosis were noted in the stroma. Spotty necrosis in the parenchyma of acinar cells, was found in 8 of 30 cases in the treated group, and one of 22 cases in the non-treated group, with a higher frequency in the former ($p < 0.05$). No significant difference was noted regarding the appearance of fatty necrosis between two groups.

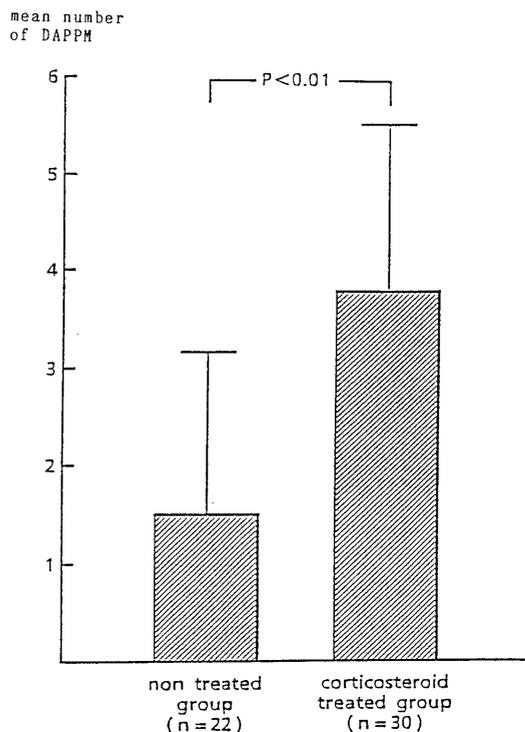


Fig. 2. Frequency of dilated acini containing PAS positive materials (DAPPM) in the corticosteroid treated and non-treated group. The frequency was expressed as the mean number of DAPPM in ten 400 power fields. A higher frequency was observed in corticosteroid treated group ($p < 0.01$).

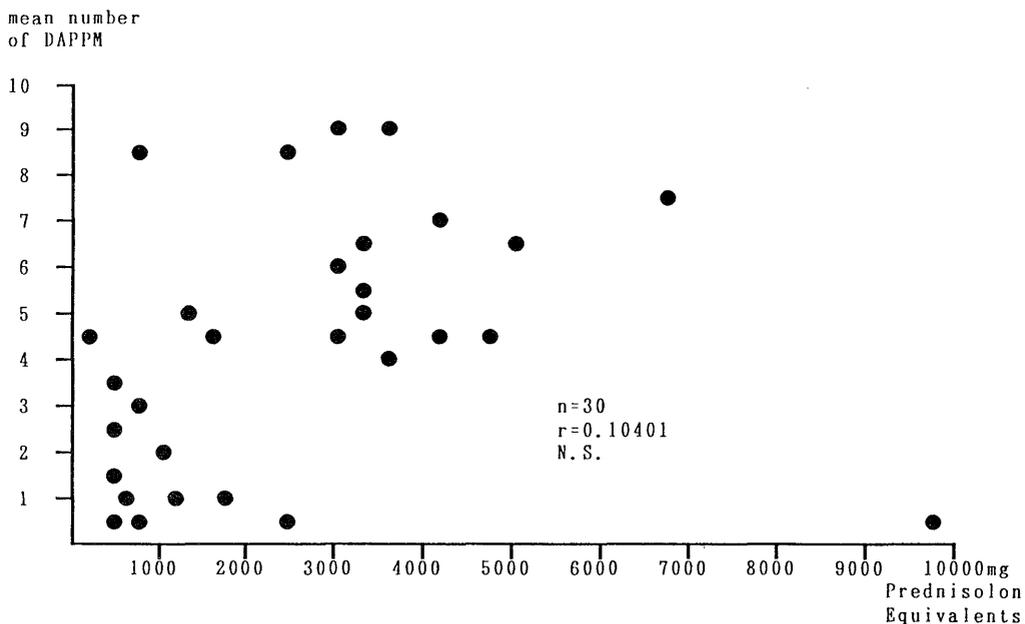


Fig. 3. Relationship between total dose of corticosteroid and frequency of dilated acini containing PAS positive materials (DAPPM). No significant correlation was noted ($r=0.10401$).

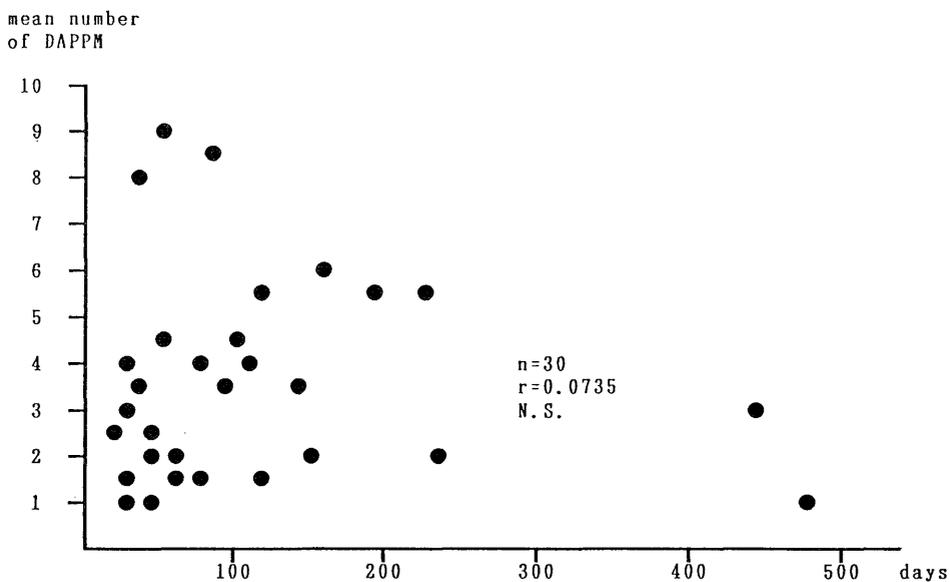


Fig. 4. Relationship between the duration of corticosteroid administration and the frequency of dilated acini containing PAS positive materials (DAPPM). No significant correlation was noted ($r=0.07354$).

2) *Appearance of DAPPM in the group treated with corticosteroid and the non-treated group*

Figure 1 shows a typical findings of dilated acini containing PAS positive materials (DAPPM). PAS positive materials were filled in the markedly dilated acinar lumen, containing lamella structures in some parts. **Figure 2** shows observed frequency of DAPPM in the group treated with corticosteroid and the non-treated group. The frequency of DAPPM was expressed as the mean observed number in ten 400 power fields. In the group not treated with corticosteroids, the frequency of DAPPM was 1.63 ± 2.0 . A higher frequency of 3.76 ± 2.6 was noted in the group treated with corticosteroid ($p < 0.01$).

3) *Total dose of corticosteroid and frequency of DAPPM*

The relationship between the total dose of administered corticosteroid and the frequency of DAPPM in 30 cases is shown in **Figure 3**. No significant correlation was noted between the total dose and frequency of DAPPM ($r = 0.10401$).

4) *Duration of corticosteroid administration and the frequency of DAPPM*

The relationship between the duration of corticosteroid administration and the frequency of DAPPM in 30 cases is shown in **Figure 4**. No significant correlation was noted between the duration of corticosteroid administration and the frequency of DAPPM ($r = 0.07354$).

5) *Comparison of other drugs administered to patients in corticosteroid treated group and*

non-treated group

Frequency of other drugs simultaneously administered to both corticosteroid-treated and non-treated patients are shown in **Table 3**. No significant difference was noted in antibiotics, antineoplastica, diuretics and hypotensive drug. H₂-receptor antagonist shows higher frequency in the non-treated group than in the corticosteroid treated group ($p < 0.05$).

6) *Electron microscopic findings observed inside of the acinar lumen in cases of corticosteroid treated group*

Figure 5 (a, b) shows electron microscopic findings. The acinar lumens were filled with filaments (F) and electron dense materials (E). Both filaments and electron dense materials were found in 6 of 9 cases (66.6%). No consistent

Table 4. Electron microscopic findings inside of acinar lumen in cases of corticosteroid treated group (n=9)

— filament and electron dense materials —

	age	sex	total dose*	filament	electron dense materials
case 1	60	F	3434mg	+	-
case 2	61	M	1260mg	-	+
case 3	31	M	10002mg	-	+
case 4	53	M	2635mg	-	+
case 5	63	M	4338mg	+	-
case 6	65	F	1120mg	+	+
case 7	61	M	3095mg	+	+
case 8	47	F	4330mg	+	-
case 9	67	F	3510mg	+	+

* total dose: calculated into prednisolon by Hollandar's method¹⁸⁾

Table 3. Other drugs administered to both corticosteroid-treated and non-treated patients

	antibiotics	antineoplastica	diuretics	hypotensive drug	H ₂ -receptor antagonist
CS treated group (n=30)	30/30 (100%)	22/30 (73.3%)	11/30 (36.7%)	1/30 (3.3%)	8/30 (26.6%)
CS non-treated group (n=22)	20/22 (90.6%)	14/22 (63.6%)	12/22 (54.5%)	1/22 (4.5%)	12/22 (54.5%)
χ^2	2.836 N. S.	0.560 N. S.	1.654 N. S.	0.005 N. S.	4.168* $p < 0.05$

* significant

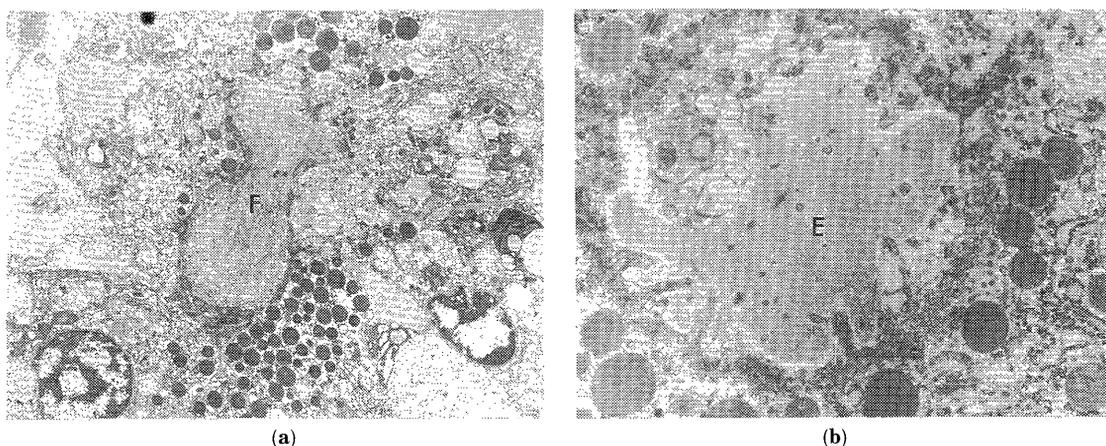


Fig. 5. Electron microscopic findings.
 (a): Filament (F) is filled in the acinar lumen ($\times 1600$).
 (b): Electron dense materials (E) is filled in the acinar lumen ($\times 3000$).

trend was noted with respect to the total dose of corticosteroid (Table 4).

DISCUSSION

The etiologic and inducing factors for pancreatitis are multiple and variable, including infection, mechanical, metabolic and nutritional, vascular, toxic, allergic and traumatic factors. Reports on drug-induced pancreatitis, however, are less frequently found than those on drug-induced hepatitis with the exception of alcoholic pancreatitis. Many aspects of the drug on the pancreas still remain unknown.

Since Stephenson, *et al.*¹⁾ in 1952 and Zion, *et al.*³⁾ in 1955 reported cases of acute hemorrhagic pancreatitis during corticosteroid administration, similar reports have continued to appear occasionally. In some of these cases, however, serious background diseases were found or other drugs with capabilities of inducing pancreatitis had also been administered, so that the concept of pancreatitis due to corticosteroids alone can not always be supported.

Stumpf, *et al.*¹⁶⁾ reported degeneration of pancreatic acinar cells, irregularly dilated acinar spaces, retention of secreted material, cellular infiltration and fibrosis in rabbits intramuscularly injected with cortisone. Bencosme, *et al.*¹⁷⁾ administered cortisone, antibiotics and vitamins in rabbits and reported similar find-

ings. Matsunga¹⁹⁾ administered prednisolon for 8 weeks in rats, and reported hypertrophy of pancreatic acinar cells by the ultrastructural quantitative observations.

Based on the study of many autopsy cases, Okumura *et al.*²⁰⁾ found morphological changes of the pancreatic exocrine system significantly more frequent in cases treated with corticosteroid than those not treated with corticosteroid.

In the present study, cases with similar background disease undergoing autopsy within a short time after death were divided into the corticosteroid-treated and the not-treated group for histopathological evaluation. Drugs simultaneously administered for the treatment of background diseases were not significantly different between corticosteroid-treated and non-treated group, except for H₂-receptor antagonist. But, administered frequency of H₂-receptor antagonist was higher in non-treated group than corticosteroid-treated group.

In the light microscopic finding, no significant difference was noted in the infiltration by inflammatory cells and fibrosis of pancreatic duct epithelium and the stroma of acinar cells.

Inside of acinar lumen, PAS positive materials ($p < 0.01$) appeared with significantly more frequency in the corticosteroid-treated group. In the pancreatic duct epithelium, goblet cell metaplasia ($p < 0.05$) and hyperplasia ($p < 0.05$)

appeared more frequently, suggesting a disturbance of pancreatic juice outflow. These findings are in agreement with those described by Carone *et al.*⁴⁾ and Wolfmüller²¹⁾, and are probably due to an increase of viscosity of pancreatic juice. Spotty necrosis of acinar cells appeared significantly more frequent in the group treated with corticosteroid. The lesions were small and more abundant in the periphery of the pancreas, suggesting disturbance of acinar cells caused by outflow disturbance of pancreatic juice described above. When the frequency of DAPPM, possibly representing one of the cause of corticosteroid-induced pancreatic damage was compared between the treated and non-treated group, a significantly larger number was noted in the treated group ($p < 0.01$).

In **Figure 3** and **4**, the relationship between the frequency of DAPPM and the total dose and duration of administered corticosteroids were showed respectively. No significant correlation was found between either pair, thus, these findings might not be ascribed to corticosteroid treatment alone. But, in case of corticosteroid-induced diabetes mellitus and gastric ulcer, onset is not always related to the total dose and duration. Therefore, the difference in individual sensitivity to corticosteroids must also be taken into consideration. The absence of such a correlation may not therefore, necessarily represent evidence against the theory of corticosteroid-induced pancreatic damage.

In a small number of cases, electron microscopic studies were carried out. Since only one autopsy material was available, the studies were limited to the inside area of the acinar lumen. The acinar lumen, as shown in **Figure 5** (a, b), was filled with filaments and electron dense materials. The electron dense materials appear to be identical with the PAS-positive materials observed under light microscopy, probably produced as the result of an increase in the viscosity of pancreatic juice. While the origin of the filament is unclear, it may represent a degradation product of pancreatic duct epithelium and pancreatic exocrine cells due to the outflow disturbance of the pancreatic juice.

In the present study, significant differences were noted regarding disturbances of the outflow of the pancreatic juice such as DAPPM,

goblet cell metaplasia and hyperplasia of epithelial cells of pancreatic duct between the corticosteroid-treated and non-treated groups. There are some compelling evidence that pancreatic damage is induced by corticosteroid, but the mechanism underlying the pancreatic damage is still unclear. Further clinical and experimental elucidation of the mechanism of corticosteroid-induced pancreatic damage should be undertaken.

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