Experimental studies on intra-abdominal adhesions

NOBORU IJIMA
M.D.

KICHI INOUE
M.D.

TAKASHI YAMAMOTO
M.D.

FUJIKO GOMI
M.D.

*The Department of Surgical Research
of The Institute for Medical Science,
The University of Tokyo, Japan*

Summary

Various intra-abdominal adhesions have been produced in rats and mice.

Those animals given an intra-peritoneal injection of protoporphyrin showed a reduction of adhesion formation.

Its mechanism has been discussed in view of Ellis's concept.

It seems to be appropriate to use PPN for patients who may develop post-operative adhesions.

Introduction

How to prevent or reduce the incidence of intra-peritoneal adhesions is one of the important problems in abdominal surgery. Adhesions of some kind develop in 70-90% of patients who have had laparotomies (Coletti & Bossart, 1964). The high incidence of adhesions and their possible serious consequences make consideration of their prevention of practical importance.

Inflammatory reactions are observed in the peritoneum following mechanical damage. Sero-fibrinous exudation produces a fibrinous coat on the damaged surface. It is considered that such fibrinous coats may cause adhesions of surrounding serosal structures. However, the fundamental etiology of the fibrinous adhesions following abdominal surgery is still poorly known. Mechanical damage, infection (Williams, 1901), reperitonealization, thermal or chemical injuries and foreign bodies (Saxen & Myllärniemi, 1968) have been discussed as causative factors of adhesions.

Recently, it was suggested by Ellis (1962) that the main factor producing postoperative intra-abdominal adhesions is tissue ischaemia. In other studies, it was found that protoporphyrin (PPN), a basic component of haemoglobin, can enter into the living cell and has an anti-anoxic action (Katto *et al.*, 1965; Yamamoto, Iijima & Katto, 1966).

The present study was designed to investigate whether PPN may prevent intra-abdominal adhesions

by eliminating the 'tissue anoxia' that was postulated by Ellis. Zinc sulphate (Pories *et al.*, 1967) reported as an effective agent in wound healing and dextran (Grosz *et al.*, 1967) which inhibits intra-abdominal adhesions by mechanical separation between intestines and steroids were also investigated.

Materials and methods

Two hundred and sixty-eight Donryu and Wistar rats and 40 DDD mice of both sexes, weighing 200-300 g (rats) and 20-25 g (mice) were used as experimental animals.

Experiment 1

A total of forty-seven rats were anaesthetized with ether. Although strict asepsis was not obtained, the abdomen was shaved and the skin painted with iodine. After laparotomy was performed by midline incision, the mesentery of the small intestine was separated closely against the bowel wall from 1.9 to 2.1 cm in length as in Ellis's (1962) experiment. These rats were divided into three groups. In the first group containing ten rats, a sheet of thin sterile polythene film was loosely wrapped around the intestinal segment of which the mesentery had been severed for a length of 2.0 cm. In the second group of eighteen rats, PPN (200 mg in 1 ml) was injected intraperitoneally every day for 5 days. In the third group containing nineteen rats, the same volume of saline was injected as in the second group. All were submitted to autopsy 5 days post-operatively to examine the extent of adhesions.

Experiment 2

A total of seventy-three rats and eighty-one mice were anaesthetized with ether. At laparotomy a part of the right parietal peritoneum was picked up and an area of about 1.5 cm² (in rats) and 0.8 cm² (in mice) was excised. In twenty-five rats and twenty-four mice the peritoneal wounds were sutured with con-

tinuous silk. In twelve rats and eighteen mice, they were massively ligated with silk. In twelve rats and twenty mice, the wounds were not sutured, but the silk was left hanging without a knot. On the next day, and the 3rd, 5th and 7th days after operation, each wound was carefully re-opened and examined for the presence of adhesion to the wounds in parietal peritoneum.

Experiment 3

A total of forty mice were anaesthetized and laparotomy was performed as described before. One part of the parietal peritoneum of each mouse was massively ligated. In the control group including ten mice 0.2 ml of normal saline was injected intraperitoneally. The other thirty mice were divided into three groups. In the first group, ten mice were given 100 mg of zinc-sulphamine-sulphate every day. In the second group, ten mice were given intraperitoneally 100 mg of Predonin 3 hr prior to laparotomy and in the third group ten mice were intraperitoneally injected with PPN (200 mg) every day for 3 days after operation. After 1 week the abdomen of each mouse was re-opened and a further exploration was made to know the extent of intraperitoneal adhesions as before. (Predonin=prednisolone-Na-succinate.)

Experiment 4

In a total of fifty-nine rats, the caecum was crushed by Pean's haemostat for 1 min to produce adhesions as shown in Grosz's experiment (Grosz *et al.*, 1967). In twenty control rats, 1 ml of saline was administered intraperitoneally. In seventeen rats and twenty rats, 1 ml of dextran and 1 ml of PPN (200 mg) were intraperitoneally injected every other day for 6 days. After 1 week each wound was re-opened and examined as before.

Experiment 5

A 2 cm midline incision was made in eighty-nine rats. In sixty-nine rats the wounds were closed as follows: peritoneum with continuous 00 plain catgut, linea alba with continuous 00 chromic catgut and silk with 0 black silk interrupted. Sixty rats were divided into three groups. The first twenty rats were injected with PPN (200 mg) daily for 7 days. In the

second twenty rats the peritoneum was not sutured and the linea alba and skin were closed as before. In the third twenty rats were used as control. After 1 week each wound was carefully re-opened and examined for the presence of adhesion to the wound. In the twenty-nine rats, if an adhesion was present it was carefully separated from the wound. Then, the wound made on the other part of an intact peritoneum was sutured as before. Sixteen out of twenty-nine rats were given single injection of PPN (200 mg) immediately after re-operation and thirteen rats were served as control. One week later, the rats were killed and development of adhesion to the initial wound was investigated.

Results

Experiment 1 (Table 1)

In seven out of ten rats in whom the intestinal segment was wrapped around with polythene film, intestinal necrosis was observed 24 hr after operation. The incidence of massive adhesions was 0% in PPN administered group and 73% in physiological saline injected group. On the other hand, the incidence of a single adhesion was 100% in the former and 0% in the latter.

Experiment 2 (Tables 2 and 3)

The incidence of adhesions 7 days post-operative appears most often in both rats and mice of which peritoneal wounds were sutured by massive ligation. It was 80 and 100% in rats and 71 or 100% in mice. These data differ remarkably from those in the peritoneum-unsutured group (26 and 20%). The group of animals in which wounds were not sutured showed the least incidence of adhesions of 30 or 33% both in rats and mice. These results are in agreement with the experimental data shown by Ellis.

Experiment 3 (Table 4)

It was indicated that the healing process of peritoneum is different from that of skin (Cameron, Hassna & De, 1957). The effect of zinc sulphate (reported as an effective agent in wound healing by Pories *et al.*, 1967) was investigated. As shown in Table 4 the incidence of adhesions in the zinc-sulphamine-sulphate treated group and PPN group

TABLE 1. Extent of adhesions following mesenteric division in Donryu rats

Treatment	No. of rats	Massive adhesions		Single adhesion	
		No./total	%	No./total	%
Control	19	14/19	73	5/19	26
Intestine was wrapped with film	10		Intestinal gangrene took place in 70% (7/10)		
PPN	18	0/18	0	18/18	100

TABLE 2. Incidence of adhesion to peritoneal wounds in Donryu rats

Treatment	No. of rats	No. with adhesions			
		After 24 hr (No./total)	After 3 days (No./total)	After 5 days (No./total)	After 7 days (No./total)
Peritoneum not sutured	24	4/5(80%)	6/10(60%)	2/5(40%)	1/4(25%)
Peritoneum sutured	25	5/5(100%)	8/10(80%)	4/5(80%)	4/5(80%)
Peritoneum ligation	12	3/3(100%)	3/3(100%)	2/3(66%)	3/3(100%)
Peritoneum not sutured only hanging silk without knot	12	0/4(0%)	0/4(0%)	0/4(0%)	0/4(0%)

TABLE 3. Incidence of adhesions to peritoneal wounds in DDD mice

Treatment	No. of rats	No. with adhesions			
		After 24 hr (No./total)	After 3 days (No./total)	After 5 days (No./total)	After 7 days (No./total)
Peritoneum not sutured	18	4/5(80%)	1/4(25%)	1/4(25%)	1/5(20%)
Peritoneum sutured	25	6/6(100%)	5/6(83%)	3/6(50%)	5/7(71%)
Peritoneum ligation	18	4/4(100%)	3/5(60%)	4/4(100%)	5/5(100%)
Peritoneum not sutured only hanging silk without knot	20	0/4(0%)	0/4(0%)	2/6(33%)	2/6(33%)

TABLE 4. Incidence of adhesion following peritoneal ligation in DDD mice

Treatment	No. of mice	With adhesions	
		No.	%
Saline control	10	10	100
Zinc-sulphamine-sulphate	10	10	100
Prednol	10	7	70
PPN	10	5	50

was 100 and 50% in contrast to 100% in the controls. Zinc-sulphamine-sulphate did not prevent intra-abdominal adhesions in this experiment.

Experiment 4 (Table 5)

It was reported that dextran (Grosz *et al.*, 1967) can prevent post-operative adhesions. As shown, Table 5, ten out of twenty-two PPN-injected mice

and nine out of seventeen mice given dextran developed adhesions. In a control group, adhesions were observed in seventeen out of twenty mice. Both dextran and PPN appear to prevent intra-abdominal adhesions equally.

Experiment 5 (Table 6)

After operation eleven out of twenty rats whose peritoneum had been sutured showed adhesions to the wound. The adhesion, comprising omentum, was easily separable from the wound. In both groups of twenty rats of which the peritoneum had not been sutured or had been sutured and injected with PPN, only five animals showed adhesions. After the second operation, only two out of sixteen rats developed adhesions to the sutured peritoneum (Fig. 1). On the contrary, nine out of thirteen control rats showed adhesions.

TABLE 5. Incidence of adhesions to pressed wounds in caecum of Donryu rats

Treatment	No. of rats	Duration of pressing (min)	No. with adhesions	
			No.	%
Saline control	20	1	17	85
Dextran	17	1	9	55
PPN	22	1	10	45

Dextran: clinical dextran (1 ml) was injected i.p. every day.

PPN: 1 ml (200 g) was injected i.p. every other day.

TABLE 6. Incidence of adhesions to laparotomy wounds in Wistar rats

	One operation			Two operations		
	No. of rats	With adhesions		No. of rats	With adhesions	
		No.	%		No.	%
Peritoneum sutured	20	11	55	13	9	69
Peritoneum sutured + PPN	20	5	25	16	2	13
Peritoneum not sutured	20	5	25			

TABLE 7. Influence of PPN on oxygen consumption of liver slices from mouse under various hypoxic conditions *in vitro* (average value in five times of experiments)

Oxygen	PPN added						
	Control	60 µg/ml		30 µg/ml		10 µg/ml	
	O ₂ /mg	O ₂ /mg	+	O ₂ /mg	+	O ₂ /mg	+
100%	11.8	11.8	0	11.8	0	11.8	0
20%	6.4	7.4	+19	7.4	+19	6.5	0
10%	3.6	4.2	+17	4.2	+17	3.7	0
5%	2.2	2.8	+27	2.6	+18	2.2	0

O₂ consumption per mg of dry weight of liver in DDD mice.

FIG. 1. (a) Intra-abdominal adhesion following laparotomy in Experiment 5. (b) Accumulation of PPN in the damaged peritoneal surface without adhesion in Experiment 5.

Discussion

Numerous clinical and experimental studies have been made of attempts to prevent or diminish the incidence of intra-abdominal adhesions. Various agents such as heparin (Lehman & Boys, 1940), corticosteroids (Eskeland, 1963), fibrinolysin (Jewett *et al.*, 1965) have been adopted for prevention of adhesions. However, they were useless or are no longer used post-operatively because of their dangerous side-effects.

Recently, Ellis (1962) has postulated a new concept—that the aetiological factor in adhesion formation is 'tissue ischaemia'. 70% of mice whose intestines were wrapped with polythene film developed gangrene of an intestinal segment by 24 hr as shown in Table 1. This suggests that intra-abdominal adhesions may be a biological adaptation, a compensatory mechanism by the host to supply oxygen-saturated blood to the damaged area as suggested by Ellis (1962).

As shown in Tables 2 and 3, intra-abdominal wounds which were closed without suturing the peritoneal defect showed a lower incidence of adhesions than those closed by suturing. This suggests that anoxia may inhibit the natural process of peritoneal healing. At this point it is interesting to note the fact that in a hypoxic condition the partially hepatectomized rat showed a delay in the initiation of mitosis (Tararico, Feller & Neville, 1969).

The aetiology of post-operative adhesions has not yet been clarified. The different influences in post-

TABLE 8. Influence of PPN on serum β -glucuronidase and acid phosphatase of DDD mouse in endotoxin shock

Enzyme activity	Control		Endotoxin		Endotoxin + PPN		Endotoxin + Predonin	
	U/mg	+	U/mg	+	U/mg	+	U/mg	+
β -Glucuronidase	1.6		2.7	70	1.8	+12	1.7	+6
Acid phosphatase	3.2		4.9	53	3.6	+12	3.6	+12

PPN (200 g), Predonin (1 mg) were injected intraperitoneally immediately after the administration of endotoxin. β -Glucuronidase was measured 1 hr and acid phosphatase was measured 2 hr after peritoneal injection of *Sh. Flexner* endotoxin (LD₅₀). The average value of ten mice.

operative adhesion formation of anoxia and trauma are too difficult to evaluate. However, it was indicated in separate papers that PPN can, to some extent, not only restore the oxygen consumption of liver slices *in vitro* (Table 7), but also prevent the increase of β -glucuronidase and acid phosphatase in the serum of the endotoxin-shocked mouse (Table 8). β -Glucuronidase and acid phosphatase are thought to be lysosomal enzymes and under hypoxic conditions their increase in serum is generally accepted. Accordingly, these facts, in addition to various experimental results already reported (Yamamoto *et al.*, 1966), suggest that PPN has an anti-anoxic effect. Moreover, Fig. 1 (a) and (b) shows that PPN accumulates in the traumatized area.

According to Ellis' concept, the effect of PPN as adhesion-preventing agent may be due to its anti-anoxic action on the damaged surface.

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