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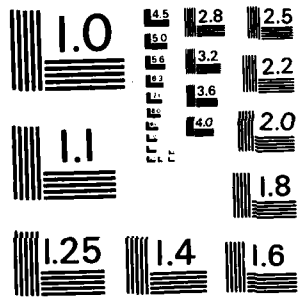
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INSTITUTE REPORT NO. 153

THE EFFECTIVENESS OF APROTININ IN BLOCKING A SHOCK FACTOR
OF PANCREATIC ORIGIN FROM THE PIG, DOG, OR MONKEY

L. WILLIAM TRAVERSO, MD, MAJ MC

DIVISION OF COMBAT CASUALTY CARE

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The Effectiveness of Aprotinin in Blocking a Shock Factor of Pancreatic Origin from the Pig, Dog, or Monkey--Traverso

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20. Abstract:

Pancreatic shock factor (PSF) causes profound hypotension when injected into the species of origin (i.e., "within species," pig PSF into the pig, etc.). Aprotinin, a proteolytic enzyme inhibitor, successfully blocked the PSF-induced hypotension "within species" in the pig but did not alter the hypotension in the dog or monkey. This "within species" variability of aprotinin blockade was further investigated by infusing PSF from either the pig, dog, or monkey into the other two species ("across species"). PSF was a hypotensive agent "across species"; aprotinin blocked the hypotensive reaction in each "across species" combination. Aprotinin is more effective as a blocking agent of PSF when infused "across" than "within species." These species differences of the aprotinin blockade should be considered when designing treatment for shock associated with proteolytic enzymes.

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ABSTRACT

Pancreatic shock factor (PSF) causes profound hypotension when injected into the species of origin (i.e., "within species," pig PSF into the pig, etc.). Aprotinin, a proteolytic enzyme inhibitor, successfully blocked the PSF-induced hypotension "within species" in the pig but did not alter the hypotension in the dog or monkey. This "within species" variability of aprotinin blockade was further investigated by infusing PSF from either the pig, dog, or monkey into the other two species ("across species"). PSF was a hypotensive agent "across species"; aprotinin blocked the hypotensive reaction in each "across species" combination. Aprotinin is more effective as a blocking agent of PSF when infused "across" than "within species." These species differences of the aprotinin blockade should be considered when designing treatment for shock associated with proteolytic enzymes.

Key Words: Pancreas; Shock; Aprotinin; Circulation; Kallikrein; Trypsin

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THE EFFECTIVENESS OF APROTININ IN BLOCKING A SHOCK FACTOR OF PANCREATIC ORIGIN FROM THE PIG, DOG, OR MONKEY

Severe systemic hypotension and portal hypertension were transiently associated with our human mixed cell pancreas autotransplants (1). Using a canine model, Mehigan and colleagues (2) suggested that the portal hypertension could be blocked with aprotinin. This drug should prove useful because aprotinin will bind three pancreatic enzymes in vitro: trypsin, chymotrypsin, and kallikrein (3,4). Unfortunately, this enzyme-binding agent is derived from bovine lung (5) and exhibits species blockade differences when tested in vivo (6). We have shown that a centrifuged supernatant from the mixed cell pancreas autotransplant (pancreatic autotransplant shock factor, PSF) exhibits all the vascular effects seen during the clinical transplants and that one of the major vasoactive agents was probably glandular kallikrein (7). PSF was obtained from the dog, pig, and monkey and was equally vasoactive in these three animals. In the "within species" experiments, the vascular effects of PSF were effectively blocked by aprotinin in the pig with as little as 2,500 kallikrein inhibitory units (KIU)/kg. Aprotinin was not effective as a blocker in the dog or monkey, even in doses as high as 10,000 KIU/kg (6).

Aprotinin and PSF were mixed in a syringe before injection to allow for the rapid binding of enzyme and inhibitor. We assumed that the species difference of the aprotinin blockade resulted either from a weaker enzyme inhibitor complex with the dog and monkey PSF, or the dog and monkey circulation contained a factor with more affinity for aprotinin than PSF. The current experiment was designed to test these two possibilities. PSF was injected "across species" to determine if one animal's PSF was vasoactive in another species, i.e., pig PSF into a dog or monkey. Each animal species PSF was found to be vasoactive in the other two species. Aprotinin was then added to the PSF before injection. Regardless of the PSF source, aprotinin could block the PSF vascular reaction when the enzyme inhibitor complex was injected "across species." For instance, when monkey PSF plus aprotinin was injected "within species" (back into the monkey) the vascular reaction was not blocked, but when monkey PSF plus aprotinin was infused into the pig or dog the vascular reaction was totally inhibited. These data indicate that the enzyme inhibitor complex was functional when injected "across species" but was probably weakened when injected "within species."

MATERIALS AND METHODS

Preparation of the Pancreatic Shock Factor (PSF)

During a sterile laparotomy under general anesthesia, a total pancreatectomy was performed on two animals of each species: pig, dog, and monkey. The pancreas was immediately immersed in Hank's Balanced Salt Solution (HBSS, Gibco). The pancreatic tissue volume was determined by volume displacement in a graduate cylinder. The pancreas was then minced to 1-mm fragments and washed three times with iced HBSS. After the final wash, iced HBSS was added to the tissue fragments so that the final volume represented one and a half times the original wet pancreatic tissue volume. Pancreatic fragments were then digested for 20 min at 37°C using 1,440 units of collagenase (Worthington Type IV, Freehold, NJ, lot #4ON075) per milliliter of wet pancreatic volume in a shaking water bath. The flask containing the incubated mixture of PSF and collagenase was immersed in ice and centrifuged at 800 g at 10°C for 5 min. The procedure required 45 min. The supernatant was decanted and frozen until used for intravascular injections.

Animal Monitoring Model

Five animals of each species were used in this study within a 6-week period: domestic pigs (16-23 kg), mongrel dogs (18-21 kg), and Rhesus monkeys (6-9 kg). The anesthesia technique was customized for each animal species to minimize cardiac depression during in vivo monitoring; the anesthesia is therefore not identical among species. Premedication was given intravenously to the dogs and monkeys and intramuscularly to the pigs in the following dosages: dogs, 4% sodium thiamylal (1 ml/6.8 kg); pigs, ketamine (0.45 mg/kg) and xylazine (0.45 mg/kg); and monkeys, ketamine (0.45 mg/kg). Pigs and dogs were then anesthetized under light endotracheal enflurane anesthesia, and monkeys were anesthetized with nitrous oxide and intravenous narcotic (fentanyl citrate (0.4 mg/ml) and droperidol (20 mg/ml), 1 ml/22 kg). Animals were placed in the dorsal position on a heating blanket; cannulae were inserted in the femoral vein for administration of normal saline and in the femoral artery to obtain blood samples and to monitor femoral artery pressure (FAP). Blood gases were measured every 30 min and the pH was kept at 7.4 ± 0.1 with respirator adjustment or intravenous sodium bicarbonate injections. After FAP and blood gases were stabilized, the abdomen was entered via an upper midline incision and a large bore polyethylene catheter (I.D.=0.23 cm, O.D.=0.36 cm) was placed into a branch of the splenic vein and advanced into the portal vein. FAP was then continuously monitored with a P2306 pressure transducer (Statham Instruments, Oxnard, CA) and recorded with a Gould Brush 2000 recorder (Gould, Inc., Cleveland, OH). The model required 30 to 45 min to prepare from premedication to recording of test data.

Experimental Design

After recording control measurements, each of the 15 animals received into the portal vein a sequence of 4 PSF injections (0.05 ml/kg) from the other 2 species. Table 1 gives an example of the injection sequence into a dog. In the third and fourth injections, 10,000 KIU/kg (6) of aprotinin (lot #L3/81, FBA Pharmaceuticals, NY) was added to the 0.05 ml/kg of PSF. Continuous hemodynamic monitoring followed each injection. The next injection in the sequence was given after control values were regained, blood gases were normal, and at least 15 min had passed.

Table 1. Injection Sequence into a Dog

| Injection # | Aprotinin | PSF ^a Source | | |
|-------------|-----------|-------------------------|-----|--------|
| | | Pig | Dog | Monkey |
| 1 | | x | | x |
| 2 | | | | x |
| 3 | x | x | | |
| 4 | x | | | x |

^aPancreatic shock factor

The FAP was recorded as the mean. To determine if PSF was vasoactive in another species, we compared the FAP before and the maximal FAP changes after PSF injection with a paired Student's t test. The ability of the enzyme inhibitor, aprotinin, to block the "across species" PSF-induced fall in FAP was analyzed with Dunnett's test for multiple comparisons (8). The maximum decrease of FAP associated with PSF alone was compared to the maximum change in FAP after the injection of PSF plus aprotinin. Aprotinin was considered an effective blocker of the PSF-induced vascular reaction if a significant difference ($p < 0.05$) was found between the PSF alone versus the PSF+aprotinin injections.

RESULTS

Each PSF isolated from one species when injected into the other two animal species produced a significant fall in blood pressure ($p < 0.01$, Fig. 1). Fig. 2 illustrates the percent change in FAP from the immediate preinjection value when PSF was intravascularly injected across species lines in combination with aprotinin. Aprotinin was an effective blocker of the PSF-induced hypotension in every instance.

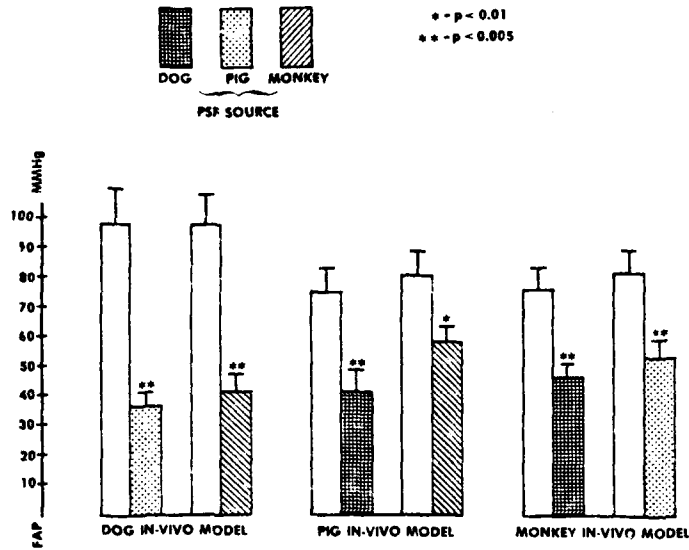


Figure 1. When 0.05 ml/kg of PSF from a dog, pig, or monkey was injected into the portal vein of the other two animal species there was a significant fall in FAP (femoral artery pressure) from pre-injection values (open bar).

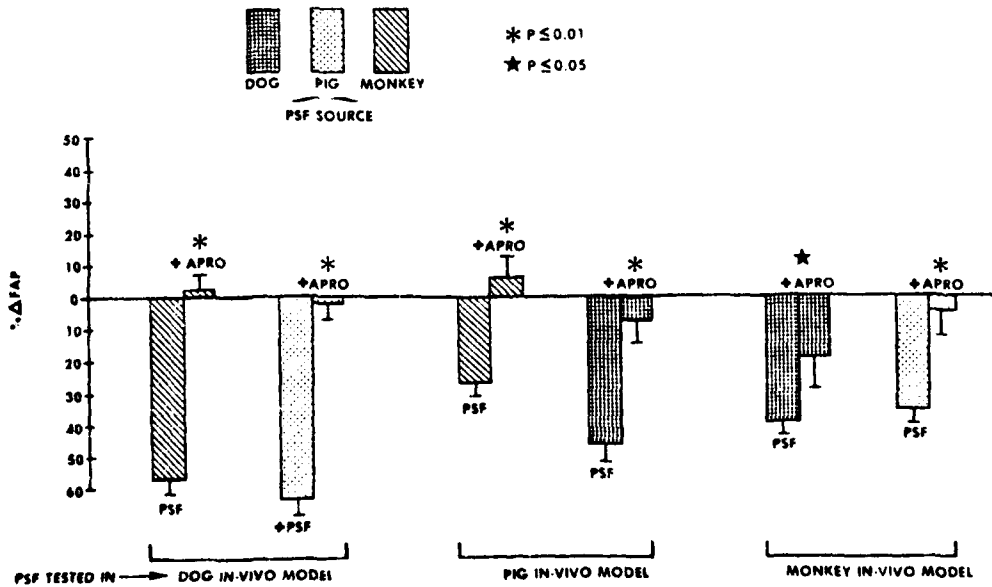


Figure 2. When PSF from a dog, pig, or monkey was injected into the portal vein of the other two species, aprotinin blocked the percent fall in femoral artery pressure (% ΔFAP) in all animals.

DISCUSSION

The hypotensive agent, PSF, is a centrifuged supernatant obtained from minced pancreas after collagenase digestion. A collagenase solution alone or minced pancreas before collagenase digestion did not produce a vascular response when tested in a canine monitoring model. The following supernatants from collagenase-digested tissues or organs also did not produce a vascular response: muscle, lung, liver, kidney, stomach antrum, duodenum, and small intestine (7). A supernatant from collagenase-digested submandibular gland produced a vascular response equal to PSF (7). Since glandular kallikrein is present in pancreas and submandibular gland, and the vascular response to PSF, submandibular supernatant, and commercially available glandular kallikrein are the same, we speculated that one of the active components in PSF was glandular kallikrein (7).

The hypotensive effect of pig PSF can be blocked in the pig ("within species") by using 2500 KIU/kg of aprotinin added to the PSF before injection, but this vascular reaction "within species" was not altered in dog or monkey using PSF from their own species, even when the dosage of aprotinin was 10,000 KIU/kg (6). Additionally, pig PSF is easily blocked in the other two species. Dog and monkey PSF are blocked with aprotinin only when the complex is injected into another animal species. The relationship of aprotinin blockade of an animal's own PSF ("within species") (6) and the results of the current study ("across species") are illustrated in Fig. 3.

APROTININ

+ PSF PARTIALLY BLOCKED * PSF COMPLETELY BLOCKED ○ PSF NOT BLOCKED

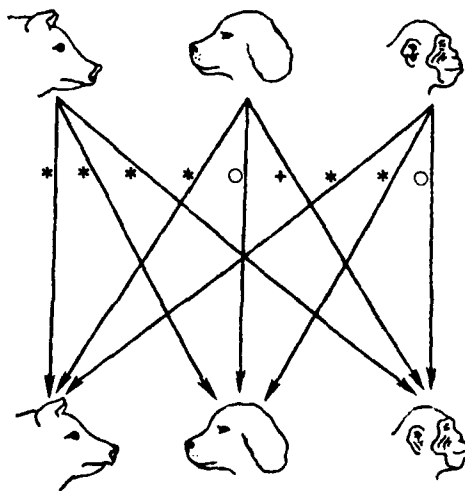


Figure 3. The "across species" blocking capability of aprotinin in this study is combined with the "within species" results of a previous study. The top figures represent the PSF source and the bottom figures represent the animal species used for the injection.

The aprotinin/enzyme complex is reversible (10) and, if other proteins are present in monkey or dog serum with a higher affinity for aprotinin, we could speculate that PSF could become uncomplexed and hemodynamically active. An agent that will block the autologous PSF in a monkey might be useful to prevent the shock associated with human pancreas autotransplantation (1) and might even be more useful than aprotinin in the treatment of acute pancreatitis.

This study points out that even though aprotinin is reported to be an inhibitor of enzyme activity in vitro (3,4) it may not be effective in vivo, and that an inhibitor of a shock agent from one animal may not inhibit a shock agent from another species. Determination of dissociation constants in vitro between blocking agents and the hemodynamically active substances (PSF, trypsin, kallikrein, etc.) only indicate possible blocking agents; these constraints do not confirm the effectiveness of the agent in a particular species.

We must examine potential pancreatic shock agents that may account for the hemodynamic changes associated with pancreatic autotransplantation when placed both in vitro and in vivo. Only then can we hope to be successful in extrapolating the results of animal enzyme inhibitor experimentation to the human clinical situation.

CONCLUSIONS

PSF was injected "across species" to determine if one animal's PSF was vasoactive in another species, i.e., pig PSF into a dog or monkey. Each animal species PSF was found to be vasoactive in the other two species. Aprotinin was then added to the PSF before injection. Regardless of the PSF source, aprotinin could block the PSF vascular reaction when the enzyme inhibitor complex was injected "across species." For instance, when monkey PSF plus aprotinin was injected "within species" (back into the monkey) the vascular reaction was not blocked, but when monkey PSF plus aprotinin was infused into the pig or dog the vascular reaction was totally inhibited. These data indicate that the enzyme inhibitor complex was functional when injected "across species" but was probably weakened when injected "within species."

RECOMMENDATIONS

Aprotinin is more effective as a hypotensive blocking agent "across species" than "within species" in the dog or monkey. These species differences of the aprotinin blockade should be considered when designing treatment for shock syndromes which may be associated with proteolytic enzymes.

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