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June 28, 1988

FINAL REPORT ON CONTRACT N00014-86-K-0496

CONTRACTOR: VIRGINIA COMMONWEALTH UNIVERSITY, SCHOOL OF BASIC HEALTH SCIENCES.

CONTRACT TITLE: EFFECTS OF oligo-PGB ON INFLAMMATION AND INFECTIOUS DISEASES.

START DATE: SEP 01, 1986

RESEARCH OBJECTIVES: To examine the anti - inflammatory effects of oligo-PGB and explore the possible modes of action and mechanisms in order to understand its anti inflammatory effects.

Progress [09/01/86- 07/31/87]

As we stated in the objective, it was paramount to establish whether oligo-PGB functioned as an anti - inflammatory agent in vivo.

Therefore, several models of inflammation, in different animal species as well as the effects of administration of oligo-PGB by different routes were tested. A summary of the in vivo anti - inflammatory effects of oligo-PGB in two different species follows.

In the first series of experiments with the C57BL/6J inbred mice, Carrageenin (20 μ l of 20 mg/ml solution) was injected into the mouse foot pad, the second foot pad was injected with an equal volume of the vehicle only (0.9% PBS + 5% NaHCO₃). The net inflammatory effects of Carrageenin was determined as the total inflammatory response minus the inflammatory effects of the vehicle. The net effects of oligo-PGB (1.35 μ g/20 μ l dose - 0.5 μ g/10 g body weight) on Carrageenin inflammation was determined from the inflammatory response following the simultaneous injection of Carrageenin and oligo-PGB minus the inflammatory response caused by injection of oligo-PGB and vehicle alone. The percent of inflammatory reduction was then calculated from these net values. Two independent methods for measuring inflammation and its reduction were used. The first was based on foot pad thickness at 1, 3 and 5 hours after injection of Carrageenin. The Dyer O.D. reader model, a spring loaded dial calliper was used to obtain the measurements, each value was based on an average of 4 measurements. The second approach was based on determination of the amount of extravasation 5 hours after injection of the irritant in the inflammation site. Radioactive I ¹²⁵ albumin (20 μ Ci) was injected I.P. one hour prior to tests, and the amount of radioactivity in the foot pad is used as a measurement of extravasation.

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Table 1 : SUMMARY TABLE

	Inflammation (mm)			Extravasation (cpm)
AVERAGE CARRAGEENIN EFFECT	0.31	0.34	0.34	28408
AVERAGE oligo-PGB EFFECT	0.21	0.28	0.26	15524
AVERAGE OF CARRAGEENIN /oligo-PGB RATIO	1.66	1.45	4.22	2.94
PERCENT REDUCTION OF INFLAMMATION	0.28	0.13	0.35	0.48

→ In the second series of experiments, the irritant injected into the mouse foot pad was a highly purified human and/or snake PLA₂s. The anti-inflammatory effects of oligo-PGB in this model system are presented in ~~table 2~~. ~~Results of the~~
discuss ~~the~~ Phospholipase A₂ Snake Venom, Syn. of fluids
inflammation reduction. (AU)

Effects of oligo-PGB on Snake Phospholipase A₂ (Naja naja) and on Purified Human Synovial Fluid PLA₂ Inflammation.

EDEMA (% of Control Sham Injected weight).		
Snake Venom alone	158 ± 3	(n = 4)
Snake Venom + oligo-PGB 20 μM	137 ± 2	"
Snake Venom + oligo-PGB 50 μM	128 ± 2	"
Human Synovial Fluid PLA ₂ alone	149 ± 2	"
Human Synovial Fluid PLA ₂ + oligo-PGB 20 μM	127 ± 5	"
oligo-PGB alone 50 μM	112 ± 1	"

The route of oligo-PGB administration was examined by testing whether orally administered oligo-PGB would be effective in reducing inflammation in the mouse paw edema model using purified PLA₂s as the irritants. Similarly, the effects of oligo-PGB in the rat carrageenin model was also tested. Results are presented in table 3 and table 4.

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Table 3

**Effect of oligo-PGB on Phospholipase A₂ -
Induced Edema in Mouse Paw Model**

MICE (n = 6)	% Increased Edema	% Protection
1. saline control 200 μ l	45.7 $\pm$ 2.1 (n=6)	0
2. 10 mg/kg oligo-PGB " oral:	35.8 $\pm$ 3.5 "	22 %
3. 100 mg/kg oligo-PGB " intubation	17.7 $\pm$ 2.0 "	61 %

Table 4

**Anti-Inflammatory Activity of oligo-PGB in
Carrageenin-Induced Rat Paw Edema**

	Foot Width (mm)	% of Control
Control	4.2 (n=10)	100%
Control + carrageenin	8.7 (n=8)	207%
Control + carrageenin + oligo-PGB*	6.4 (n=5)	152%

* oligo-PGB (1.5 mg/kg) injected IP 1 hr prior to irritant

These results clearly establish the anti - inflammatory effects of oligo-PGB. Most importantly these data show the effectiveness of oligo-PGB administered by the oral route.

Our previous studies had demonstrated conclusively that oligo-PGB 29-II inhibited human, non pancreatic, Ca²⁺-dependent phospholipases A₂ (PLA₂s) active at neutral pH, and the inositol-specific phospholipase C. The PLA₂ isolated from various human sources (including neutrophils, platelets, plasma, synovial fluid) was approximately an order of magnitude more sensitive to inhibition by oligo-PGB; IC₅₀ = 4-8 μ M, PLA₂ vs 50-100 μ M, PLC. In addition we were able to show that oligo-PGB had potent anti-oxidant activity (figure 1) using air exposure of phospholipid as a model of auto-oxidation. The anti-PLA₂ and anti-oxidant properties of oligo-PGB in vitro indicated that these polymers may be cytoprotective, could influence the arachidonic acid cascade, and therefore could function as anti-inflammatory agents.

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Studies with prelabelled human endothelial cells in culture and prelabelled human neutrophils have subsequently shown that oligo-PGB inhibits ionophore (and histamine) stimulated mobilization of arachidonic acid from membrane phospholipid (table 5); and this is reflected by not only depressed levels of unesterified arachidonate but also decreased levels of prostaglandins and leukotrienes in general (not shown).

Table 5

**Inhibition of Arachidonate Mobilization
by oligo-PGB Using Prelabelled Human Neutrophils**

Preincubation oligo-PGB Albumin		Control (C)	Percent inhibition	A23187	C - S (S)	Percent inhibition
0	5 μ M	3,518	-	10,668	7,150	-
10 μ M	5 μ M	2,459	30.1%	5,598	3,139	56.1%
20 μ M	10 μ M	2,301	34.6%	4,721	2,420	66.2%
30 μ M	15 μ M	2,692	23.5%	5,399	2,707	62.1%
40 μ M	20 μ M	2,647	25.3%	4,374	1,746	75.6%
10 μ M	20 μ M	3,109	11.6%	11,968	8,859	no effect

SUMMARY:

The results have establish in-vivo the anti - inflammatory effects of oligo-PGB.

The biochemical data, provide a fundamental understanding of how oligo-PGB can protect various cells and organ systems from injury by focusing on biochemical events at the level of the cell membrane. The in vitro studies illustrating anti-lipolytic and anti-oxidant activities of oligo-PGB, and the confirmation of these effects on cellular signal and transduction events as measured by depressed arachidonate mobilization and prostanoid formation provide a molecular basis for the anti-inflammatory and cytoprotective action of oligo-PGB.

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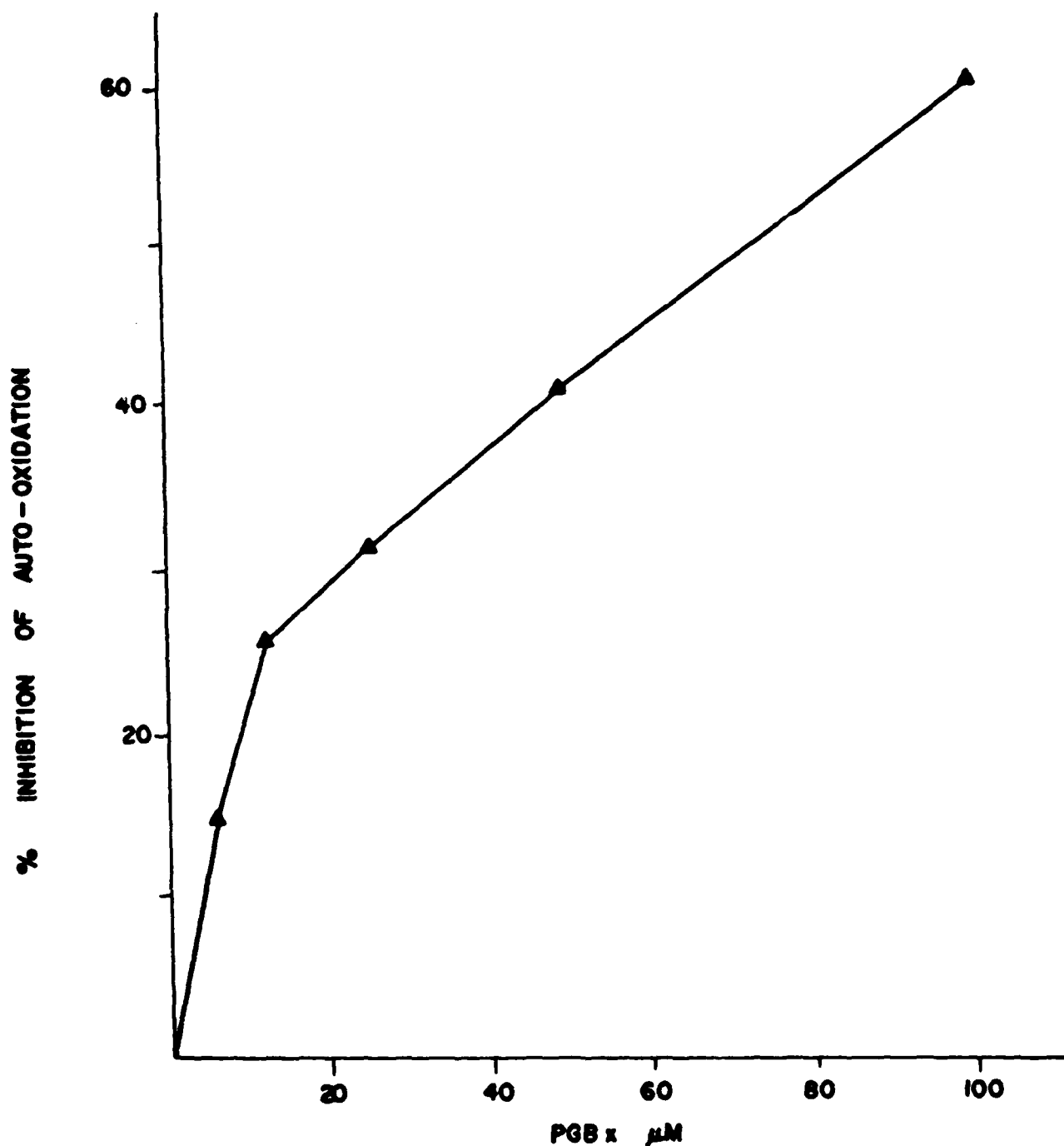


Figure 1 Inhibition of Auto-Oxidation of Phosphatidylethanolamine
by PGBx