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ANTIALLERGIC ACTIVITY OF SOME NOVEL OLIGOPEPTIDES RELATED TO IMMUNOGLOBULIN E

Bijoy Kundu*, Manisha Shukla, Sanjay K.Khare
Division of Biopolymers
Prem P. Gupta, Rashmi Singh, Amarnath, Gyanendra K. Patnaik
Division of Pharmacology
Central Drug Research Institute, Lucknow 226001 India

Abstract: Five analogues of Ala-Asp-Ser-Asp-Gly-Lys related to IgE with modifications at position 1,2 and 6 have been evaluated for antiallergic activity. One of the peptides (CDRI compound 94/335) was found to exhibit significant activity by oral route in rats. © 1997 Published by Elsevier Science Ltd.

Bronchial asthma is a disease of hyperirritable airways manifested by reversible airway obstruction associated with bronchospasm and mucous secretion. Although various pharmacological agents have been investigated, the drug of choice todate remains limited to prophylactic agents which simply provide rapid control of symptoms of asthma. However, the high incidence of toxicity is the major side effects associated with these class of drugs. The search for a curative, nontoxic and potent drug is inevitable in the treatment of asthma.

Direct correlation between IgE titres and the distribution of mast cell and basophils which bear high affinity receptors for IgE has attracted the attention of many investigators[1-4]. The mast cell bound Ig when reacts with antigen there is an explosive discharge of preformed mediators which account for many of the symptoms of allergic diseases[2-5].

Recently Noguchi et al[6] reported a new class of oligopeptides related to the sequence of IgEFc fragment (330-334)[7] which exhibited very high order of biological response by inhibiting the production of IgE antibody. One of their hexapeptides Ala-Asp-Ser-Asp-Gly-Lys exhibited marked improvement in the biological activity over that of the Hamburger's pentapeptide[7] in blocking the allergic response. In this vein, development of new synthetic peptides with increased potency may lead to the development of therapeutically useful antiallergic agent.

CDRI Communication No. 5524

In the present paper antiallergic activity of novel analogues related to Ala-Asp-Ser-Asp-Gly-Lys, viz. D-Ala-Asp-Ser-Asp-Gly-Lys (**1**), MeAla-Asp-Ser-Asp-Gly-Lys (**2**), Ala-Glu-Ser-Asp-Gly-Lys (**3**), Ala-Asp-Ser-Asp-Gly-Lys-NH_2 (**4**) and Ala-Gly-Ser-Asp-Gly-Lys (**5**) [CDRI Compound No. 94/335] are described.

Table 1 Physicochemical Characteristics of various peptides

Peptides	[α] D (in MeOH)	FAB MS (M+H)	Rt (min)	Amino acid analysis*
Ala-Asp-Ser-Asp-Gly-Lys (Lead Peptide)	-24.00;c,0.10	592	16	D 3.09 (2), S 0.91 (1) G1.04 (1), K 1.06 (1), A 1.00 (1)
D-Ala-Asp-Ser-Asp-Gly-Lys	-47.62;c,0.06	592	18	D 2.1 (2), S 0.93 (1) G 1.03 (1) K 1.04 (1), A 0.98 (1)
MeAla-Asp-Ser-Asp-Gly-Lys	-39.00;c,0.10	606	17.5	D 2.06 (2), S 0.97 (1), G 1.04 (1) K 0.98 (1)
Ala-Glu-Ser-Asp-Gly-Lys	-10.00;c,0.08	606	16.4	D 0.98 (1), S 1.01 (1), G, 1.06 (1) E 0.95 (1), K1.04 (1) A 1.04 (1)
Ala-Asp-Ser-Asp-Gly-Lys-NH_2	-22.39;c,0.07	591	19	D 2.07 (2),S 0.92 (1), G1.00 (1), K 1.04 (1), A 1.07 (1)
Ala-Gly-Ser-Asp-Gly-Lys	-13.24;c,0.07	534	18.6	G 2.09 (2),S 0.91 (1), D0.98 (1) K 1.06 (1), A 0.98 (1)

*Amino acids have been represented by single letter code.

The peptides were synthesised by solid phase method[8] and purified to more than 98% homogeneity using a sephadex G10 column followed by reverse phase HPLC[9]. As expected the crude peptides exhibited 5-20% of aspartimide as byproduct due to the presence of Asp-Gly and Asp-Ser which are known to be one of the most prone sequences for aspartimide formation. This was evident by mass spectrum of crude peptides exhibiting an additional peak 67u larger than that of the required peptide. This mass difference suggested that unknown peak was caused by aspartimide formation (-18u) and subsequent ring opening by piperdine adduct (+85u). Table I shows the physicochemical data of five synthetic peptides.

In the first instance, antiallergic activity of synthetic peptides was evaluated by studying anti-PcA[10] (passive cutaneous anaphylaxis) and mast cell stabilising activity[10] by intraperitoneal routes in rats. The results have been summarised in tables 2 and compared with disodium cromoglycate (DSCG), a standard antiallergic drug used clinically. Analogues 1,2,3 and 4 retained 60-90% activity of the lead peptide Ala-Asp-Ser-Asp-Gly-Lys in anti-PCA test while they were equipotent to the lead peptide in mast cell stabilising assay.

Analogue 5 on the contrary, at 1 mg/Kg in anti-PCA test was equipotent to both, the lead compound at 1mg/kg and DSCG at 50 mg/Kg. However, in the mast cell stabilising assay peptide 5, at 1 mg/Kg was more active than the lead peptide but was equipotent to DSCG at 50 mg/Kg. Thus peptide 5 appears to be atleast 50 times more potent in terms of dose per dose of DSCG. Further, the high order of antiallergic activity exhibited by peptide 5 clearly suggests that the Asp at position 2 is not essential for activity and can be replaced with Gly. This finding is of great significance from the point of view that one of the troublesome sequence Asp-Ser in the lead peptide can be replaced with Gly-Ser which in turn will bring about substantial decrease in the aspartimide formation. This was evident during synthesis of peptide 5, in which aspartimide formation as byproduct was found to be as low as 5% compared to 20% for the lead peptide.

Table 2: Antiallergic activity of compounds by intraperitoneal route

Compound	Percent protection of PCA at 1mg/kg, i.p. (Mean± SE;n=4)	Percent protection of mast cell degranulation at 0.25 mg/kg, i.p.x 4 days(Mean ± SE; n=4)
Ala-Asp-Ser-Asp-Gly-Lys	75 ± 2.92	64 ± 2.78
D-Ala-Asp-Ser-Asp-Gly-Lys (1)	64 ± 2.40	61 ± 3.02
MeAla-Asp-Ser-Asp-Gly-Lys (2)	67 ± 2.72	60 ± 2.88
Ala-Glu-Ser-Asp-Gly-Lys (3)	45 ± 2.78	63 ± 2.54
Ala-Asp-Ser-Asp-Gly-Lys-NH_2 (4)	68 ± 2.80	61 ± 1.73
Ala-Gly-Ser-Asp-Gly-Lys (5)	78 ± 2.86	78 ± 3.12
DSCG	77 ± 2.45*	84 ± 4.21*

*DSCG was administered at 50 mg/kg

Now since parenteral administration receives poor patients compliance, particularly for a chronic disease like asthma which requires long term drug administration. A simple, painless, economical and practical method will be oral administration. Therefore, it was decided to determine the antiallergic activity of lead peptide and of analogue 5 when administered orally in rats as an indication of its potential use in humans. The results have been summarised in table 3. Both the peptides showed biological activity in a dose dependent manner. Maximum biological response was observed at 7.5 mg/kg *p.o.* In anti-PCA test peptide 5 exhibited significant inhibition of PCA with ED_{50} value of 0.6 mg/Kg which was close to 0.9 mg/Kg for the lead peptide. However, in mast cell stabilising assay peptide 5 exhibited much better protection of mast cells and was determined to have ED_{50} value of 1 mg/Kg, compared to 3 mg/Kg for the lead peptide.

Table 3: Antiallergic activity of compounds by oral route

Compounds	Dose mg/kg	% Protection of PCA (Mean ±SE, n =4)	Dose mg/kg x 5days	% Protection of Mast cells (Mean ±SE, n=4)
Lead peptide	0.5	44±2.64	0.1	34±4.51
	1.0	52±2.81	0.2	41±4.72
	2.5	67±2.68	0.5	47±3.12
	5.0	75±3.10	1.0	72±4.10
	7.5	86±3.22	2.5	79±3.81
	10.0	89±3.80	-	-
Peptide 5	0.5	47±2.45	0.1	39±1.00
	1.0	57±1.45	0.2	50±4.01
	2.5	69±2.78	0.5	70±3.81
	5.0	77±3.22	1.0	80±4.30
	7.5	88±3.82	2.5	87±4.60
	10.0	90±4.20	-	-

Our studies thus suggest that compound 5 with Gly at position 2 exhibited significant protection of PCA and mast cells at much lower dose in comparison to DSCG. Latter is however associated with a major drawback as it has a very poor oral absorption and is administered only through an aerosol. Further, repeated administration of DSCG has also shown tachyphylaxis. Therefore, increasing interest has been shown in developing orally active antiallergic agents. Analogue 5 in this direction may have a potential as a therapeutic drug as it will be nonallergenic and inexpensive to manufacture.This study provided first experimental evidence for the existence of antiallergic activity in small peptides by oral route and opens a new avenue for further exploration.

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References & Notes

1. Stanworth, D.R.; Hamphrey, J.H.; Bennich, H.; Johansson, S.G.O.; Lancet, 1968, 17.
2. Sptiz, E.; Gelfand, E.W.; Sheffer, A.L; Austin, K.F.; J of Allergy and Immunology, 1972, **49**, 337.
3. Kalimer, M.A.; Hargreave, F.E., ed. Airway reactivity Mississanga, Ontario, Canada, Astra Pharmaceuticals Canada Ltd. 1980 p. 175.
4. Kalimer, M., Chest, 1985, **25**, 875.
5. Bennich, H.; Johansson, S.G.O.; Progr. Immunol, 1874, **1**, 49.
6. Noguchi, K.; Matzuo, K.; Ohta, N.; Chem. Pharm. Bull. 1990, **38**, 2463.
7. Hamburger, R.N.; Science, 1975, **189**, 389.
8. The peptides were synthesised by Fmoc/But strategy on wang's resin except peptide **4** which was synthesised on MBHA resin.
9. C_{18} reverse phase HPLC was performed. Buffer a contained 5% acetonitrile in 0.045% TFA, buffer B contained 50% acetonitrile in 0.037% TFA. Peptides were eluted with a 5-30% buffer B linear gradient over 60 min at 0.5 ml/min, monitered at 212 nm.
10. Gupta, P.P.; Srimal, R.C.; Srivastava, M.; Singh, K.L.; Tandon, J.S.; Int. J. of Pharmacognosy, 1995, 33, 70.

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