

REVIEW

The chromones: history, chemistry and clinical development. A tribute to the work of Dr R. E. C. Altounyan

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Chromones

Chromone is the chemical term for the chemical formula 5:6 benz-1:4-pyrone (Fig. 1). The collective term 'chromones' is used for chemical compounds that contain this structure, some of which are used in clinical medicine. The chromone structure is found within the chemical structure of the flavonoids, a group of naturally occurring substances that are of current interest because of their antioxidant activity.

The first chromone to be used in pure form in clinical practice was khellin (Fig. 2), extracted from the seeds of the plant *Ammi visnaga* (Fig. 3). *Ammi visnaga* grows wild in many eastern Mediterranean countries and decoctions of its seeds have been used for centuries as a diuretic and as a smooth muscle relaxant, particularly for the relief of ureteric colic. Khellin was first prepared in impure form by Mustapha in 1892 [1]. Pharmacologically it was studied by Samaan in 1932 [2] who showed that it relaxes all visceral smooth muscles by direct action on the muscle fibres. The main active ingredient was isolated in its pure form by Ernst Späth in Vienna in 1934 [3]. He also established the structure analytically. Khellin was first synthesized by Dr (later Professor) G. R. Ramage in conjunction with R. A. Baxter and N. R. Timson at the British Schering Research Institute at Alderley Edge in Cheshire, UK in 1947 [4].

Also in 1947 Anrep *et al.* [5] reported another usage of intramuscular khellin; providing complete and prolonged relief in bronchial asthma.

The 1952 edition of The Extra Pharmacopoeia of the United Kingdom lists two pharmaceutical products of khellin – Benecardin from Bengers and Eskel from Smith, Kline & French. Both were tablets, used as smooth muscle relaxants and coronary artery dilators. The German 'Rote Liste' of 1954 and 1957 lists a 0.5% solution of khellin (Khelfren®) which was used as an aerosol for asthma.

It is likely that the 1947 Lancet paper by von Anrep that

stimulated the research group at Bengers's Research Laboratories in Holmes Chapel, Cheshire UK, to start a programme in the early 1950s to synthesize and develop soluble forms of khellin that could be administered either orally or by inhalation for the treatment of asthma.

Developing the early chromones

One of the first chromone analogues to be produced based on khellin was designated K18 (Fig. 4). In a series of unpublished experiments in 1954 at Bengers pharmacology department, Philip Sheard showed that fatal bronchoconstriction in guinea pigs induced by an aerosol of 1% histamine or 2% methacholine was prevented by the inhalation of a 1% aerosol of K18. Sheard and his coworkers used the 'Herxheimer' guinea-pig [6] in which guinea-pigs were sensitized to egg albumin and then challenged with a bronchial aerosol of egg albumin; the ability of new compounds given prior to the challenge to prevent the bronchoconstriction was then tested.

It was known that the immediate bronchoconstriction was primarily caused by the release of histamine, but the work of Dr W. E. Brocklehurst [7,8] had shown that another factor — SRS-A (slow releasing substance of anaphylaxis) was involved. In a series of experiments using sensitized guinea-pigs challenged with antigen, he was the first to differentiate between the effects of histamine and SRS-A. He also showed that SRS-A was not 5-hydroxytryptamine, bradykinin or substance P and that guinea-pig and human SRS-A had identical pharmacological properties [7,8].

At Bengers, research chemists began to synthesize more chromones and the pharmacologists investigated their ability to prevent the bronchoconstriction induced in the lungs of an egg albumin-sensitized and -challenged guinea-pig. However, difficulties arose with the interpretation of these early experiments; it was soon realized that these compounds, as well as having antihistaminic and anti-SRS-A activity also inhibited the formation of these substances in the challenged, sensitized lung.

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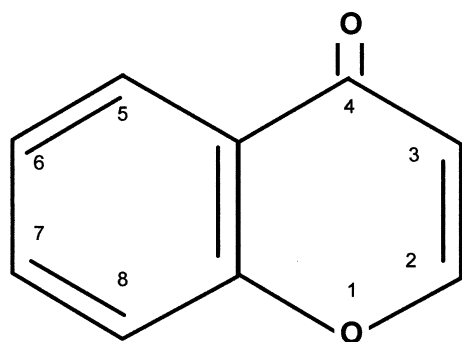


Fig. 1. The chromone chemical structure.

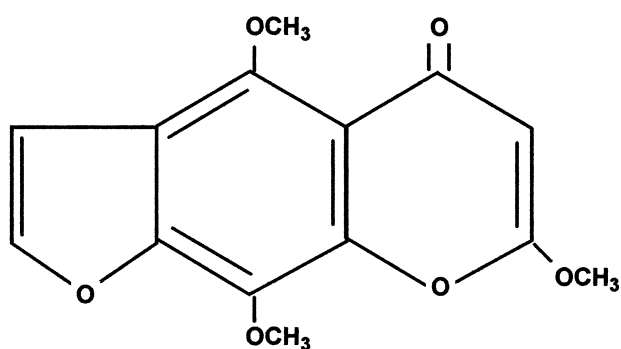


Fig. 2. Chemical structure of khellin.



Fig. 3. *Ammi visnaga*.

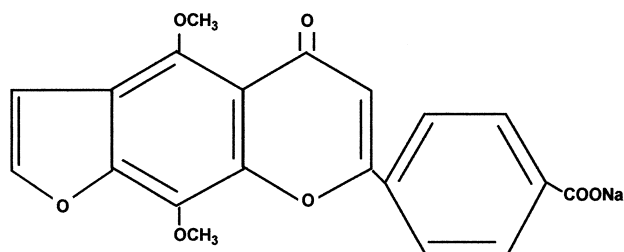


Fig. 4. Chemical structure of the first of the soluble chromones, K18

Subsequently Sheard and Blair were the first to report that a chromone, sodium cromoglycate, reduced the antigen-induced release of both histamine and SRS-A from passively-sensitized human-chopped lung [9]. This paper was published in 1970.

But the original brief given to the research group had been to develop a more potent bronchodilator, and the possible significance of the results of the inhibition of the release of the mediators of the allergic reaction was ignored until it was recognized by a medical liaison officer newly appointed to Bengers's Laboratories in 1956, Dr Roger E. C. Altounyan.

Dr R. E. C. Altounyan: his early life and career

Roger Altounyan was born in Aleppo, Syria in 1922 and was educated at Abbotsholme School, Derbyshire in the UK.

He intended to follow his father Dr Ernest Altounyan to read medicine at the University of Cambridge, but the Second World War interrupted his studies.

In 1946, after a distinguished wartime career as a bomber instructor pilot for which he was awarded the Air Force Cross, he commenced his preclinical medical studies at Emmanuel College Cambridge followed in 1949 by clinical studies at the Middlesex Hospital Medical School in London.

After qualification in 1952, he returned to Aleppo to join his father and grandfather in running the Altounyan Hospital, which his grandfather had founded.

These were very difficult times politically in Aleppo, and in 1955 Altounyan (Fig. 5) returned to England and sought a job, a training post in the National Health Service, but found there were too many senior registrars chasing too few consultant posts. He and Dr Leon Goldberg — who had become the Research and Development Director at Bengers — had been medical students together at Cambridge, so in 1956 he was invited to join Bengers Laboratories as a medical liaison officer with ill-defined responsibilities.

Roger Altounyan was atopic and as a child had suffered from severe eczema and mild attacks of wheezing. His first severe attack was of nocturnal asthma when a medical student and progressed to become severe chronic asthma,



Fig. 5. Dr Roger Altounyan in the 1950s.

which he treated with inhaled adrenaline plus atropine methonitrate and intermittent oral corticosteroids.

Initially his responsibilities at Bengers were concerned with the evaluation of an iron/dextran complex injection for the treatment of iron deficiency in both animals, Imposil and in humans, Imferon. He was also involved in the development of a proteolytic enzyme treatment for chronic bronchitis, Lomudase. In mid-1957, Dr W. Robinson, consultant chest physician at Monsall Hospital in Manchester invited Altounyan to start a clinic to follow up asthmatic inpatients after hospital discharge. In 1958 they published a paper in the *Lancet* [10] in which they reported on the administration of desoxyribonuclease and chymotrypsin to chronic bronchitic patients using a dry powder inhalation device invented and developed by Altounyan (Fig. 6). This eventually became the Spinhaler.

Although he was not part of the research team developing the chromones he took a great interest in the work they were doing. Although their aim was to look for new bronchodilator drugs, he realized the potential of the ability of these compounds to inhibit allergic reactions. He also recognized the limitations and problems of animal models in asthma not

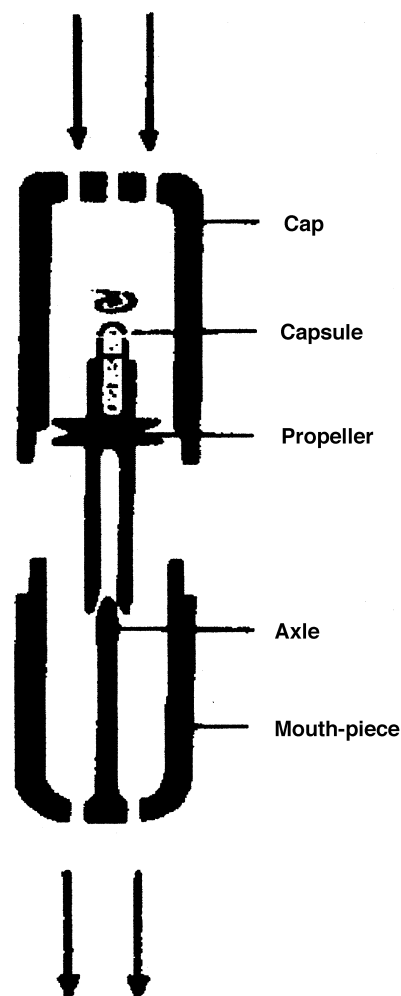


Fig. 6. Drawing of first Spinhaler invented by Dr R. E. C. Altounyan. Reproduced by permission [10].

least because of the potency of the antihistamines in protecting guinea-pigs whilst having no significant effect upon his own asthma.

Altounyan therefore decided to investigate the potential of the new khellin-derived compounds on a human model; he began to perform antigen inhalation bronchial challenges on himself and tested the ability of the prior administration of new compounds to prevent the induced asthmatic reactions.

His first documented bronchial self-challenge was on July 18th 1957, using an extract of guinea-pig hair. On 5 August 1957, he showed that pretreatment with 50 mg of intramuscular mepyramine gave very limited protection against this challenge. This supported the view that bronchoconstriction in man induced by inhaled antigen challenge was unlikely to be caused by histamine alone and that SRS-A was also involved; so he embarked on a series of experiments in which he treated himself with an antihistamine plus one of the synthesized chromones. Figure 7 shows the results of

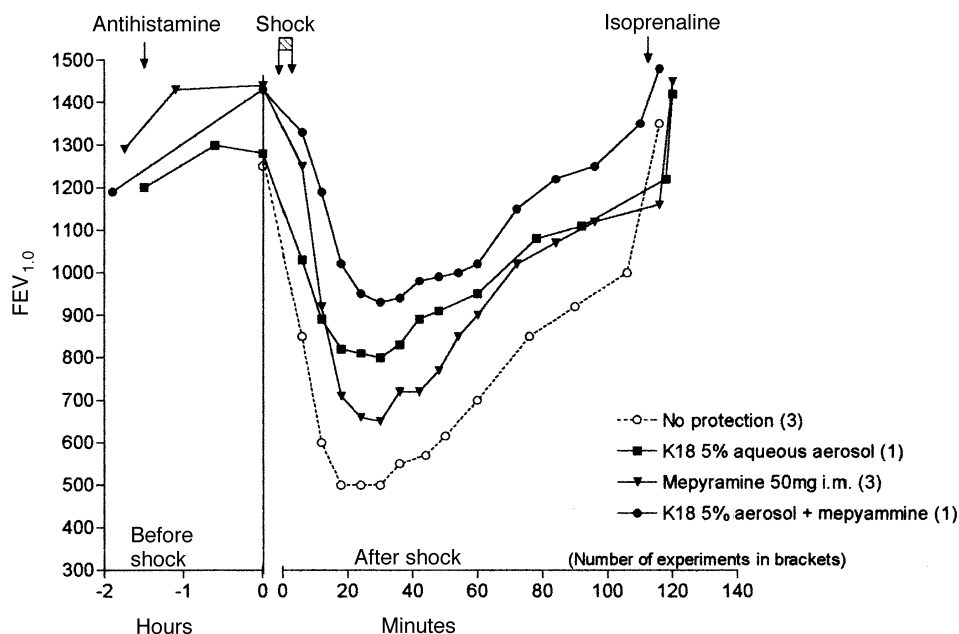


Fig. 7. The results of a series of bronchial challenge experiments carried out by Altounyan in 1958. No protection (3) = the mean results of 3 challenges with inhaled antigen alone. K18 5% aqueous aerosol = the effect of pretreatment with K18 inhaled as a 5% aqueous aerosol administered 30 min before antigen challenge. Mepyramine 50 mg i.m. (3) = the mean results of three inhaled antigen challenges pretreated with 50 mg of intramuscular mepyramine administered 1.5 h before challenge. K18 5% aerosol + mepyramine (1). The effects on antigen challenge of inhaled K18 and IM mepyramine administered together.

a series of challenges carried out during the first 6 months of 1958, in which he pretreated himself with either intramuscular mepyramine or inhaled K18, with both or with nothing (no protection) before the challenge with inhaled guinea-pig hair.

In the guinea-pig model K18 had been shown to be a bronchodilator and a specific antagonist of SRS-A but with no antihistaminic activity. It had no effect on established SRS-A-induced contraction but had a highly specific protective action against SRS-A-induced spasm. In his own self-challenges Altounyan showed that K18 alone did provide some protection against the antigen challenge (shock), that mepyramine alone did not but the two together gave quite good protection — about 40%.

Altounyan tested K18 on 25 outpatients. He showed that in a subgroup of patients that had shown some response to antihistamine therapy, adding K18 enhanced this response — he concluded this was due to K18's anti-SRS-A effect. However, K18's development was discontinued after it was shown to have a possible hepatotoxic effect, with an increase in serum bilirubin levels in some patients.

A setback in chromone development

The direction of the research project changed from trying to find a better bronchodilator to following up Altounyan's

hunch. It was possibly the difficulties in trying to correlate the results from animal and human experiments together with the lack of confidence in this medical liaison officer untrained in research, that led to the chromone project being stopped in 1961 by Bengers' newly appointed Research Director. Understandably he requested a full review, but then attempted to sell the project to another company!

However, the main objections came from an external 'world expert' who pronounced that 'Altounyan results were quite useless and unproven and that even if proven such a drug would have no place in the market' (Altounyan; Freeman Oration, Charles Blackley Symposium, 1976).

Most of the departmental heads that had led the chemical and pharmacological programmes left and Altounyan was forbidden access to the research group. This was an unfortunate decision, as he and the research group were beginning to understand the structure–activity relationships and development work on two potentially useful chromones was stopped. These were BLA 8 and GR4 (also designated BLA 13. GR4 (Fig. 8) and had been synthesized and given to Bengers by Professor George Ramage.

Up to the time of the Bengers internal review, Altounyan had carried out 150 documented bronchial challenges on himself and had tested 50 different chemical compounds.

Tables 1 and 2 are taken from the report of the internal review and provide details of tests carried out on 14 of these

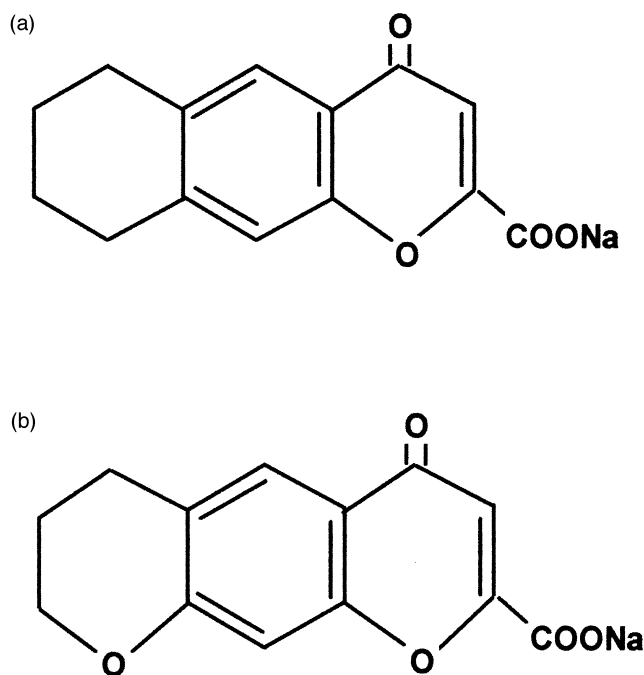


Fig. 8. Chemical structures of (a) BLA 8 and (b) BLA 13 (GR4).

compounds. Table 1 gives the structures of the 14 compounds and the results of Altounyan's own experiments, including then taste and irritancy, bronchoconstricting or bronchodilating effect after inhalation and the protective effects against antigen challenge when inhaled at different times before challenge. Table 2 provides details of the results of the experiments carried out in animals — the inhibition of the release of histamine and SRS-A in the lungs of sensitized and challenged guinea-pigs, their effects as specific antagonists and the LD₅₀. The anaphylactic release of histamine was studied by the method of Austin and Brocklehurst [11] and the SRS-A assay used the method published by Chakravarty [12].

In his own appraisal of the project, Altounyan stated that: 'On the basis of human studies a clear structure-activity relationship is apparent. Chromone-2-carboxylic acid derivatives have by far the greatest activity of the compounds

tested, the most active of these compounds being BLA 8, 13, 14, 80 (Fig. 9) of which BLA 8 is probably the best. In this group of compounds definite structural activity has been demonstrated. For example, reduced activity occurs where ring A is cyclopentane or methylenedioxy, if ring A is opened and replaced by alkyl or alkoxy groups at C6 or C7, or ring A is moved to either 5,6 or 7,8 positions. Activity is also reduced by opening ring C, substitution of a methyl at ring C, replacing ring C by a carboxy-3-methylfurano ring or replacing the 2-carboxy group by an ester or amide group.'

These observations held true up to the discovery of sodium cromoglycate in 1965 and of all active chromones since.

BLA13 (GR4) inhibited the release of both histamine (43% inhibition at a concentration of 2660 µg/mL) and SRS-A (90% at 2660 µg/mL) in the chopped guinea pig lung challenge test. This was the first compound that had no inherent bronchodilator activity, yet gave 70% protection in a bronchial antigen challenge experiment carried out in August 1959 by Altounyan on himself. Subsequently, he showed that it gave 56% protection given 3 h before challenge and 43% at 6 h before.

BLA13 (GR4) was also the first chromone to be used in a multipatient, bronchial antigen challenge trial. On five occasions between March and July 1960, 10 patients attending the chest department at London's Brompton Hospital were challenged with inhaled antigen; inhaled GR4 was given 2, 4 and 6 h before challenge on three of these occasions.

In a statistical analysis by Dr P. Armitage, GR4's mean protection against antigen challenge was 44% at 2 h (95% CI 2–85), 45% at 4 h (95% CI 6–85), and 63% at 6 h (95% CI 24–101).

This report was apparently ignored; Altounyan himself criticised the results since he did not agree with the technique of bronchial antigen challenge used at the Brompton Hospital at that time. This was to give large and variable doses of inhaled antigen, record the change in FEV₁ once only at 5 min after challenge and then reverse the bronchoconstriction with either inhaled isoprenaline or intramuscular adrenaline. The mean fall in FEV₁ in the control challenges in the Brompton trial was 56% (range 24–76%).

BLA 8 was first used by Altounyan in a self-challenge experiment in October 1960, giving up to 70% protection

Fig. 9. Chromone structures that provide optimal protection against bronchial challenge. Reproduced from a report written by Dr R. E. C. Altounyan in 1961.

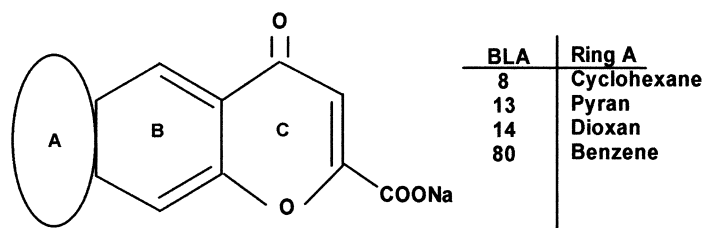


Table 1. Results of human bronchial challenge experiments on 14 compounds carried out by Dr. R. E. C. Altounyan 1956 and 1961

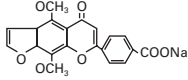
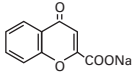
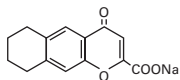
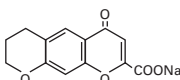
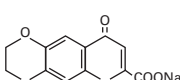
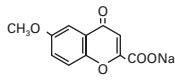
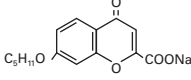
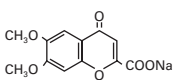
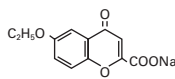
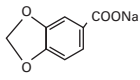
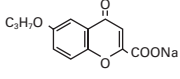
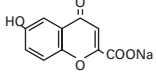
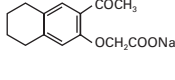
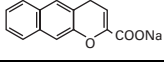
Compound	Formula	Toxicity of 5% aqueous aerosol		Human prophylactic experiments % protection/no of trials		
		Taste/irritancy	FEV ₁ change/min	1 h	3 h	6 h
K18		+/+	+10/30	50/1		
BLA 1		0/0	0/20	30/2		
BLA 8		0/0	0/20	> 70	57	30
BLA 13		0/0	0/30	> 60	56/1	43/1
BLA 14		0/0	0/30	> 70	41/1	55/1
BLA 15		0/0	0/20	45/1		
BLA 18		0/++++	−20/1	20/1		
BLA 20		0/0	0/30	60/1		25/1
BLA 21		0/0	0/20	50–58/2		
BLA 25		+/0	0/20	23/11		
BLA 30		0/0	+10/20	47/1		
BLA 50		0/0		< 35/1		
BLA 68		0/++	−8/20	37/1		
BLA 80		0/0	0/20	> 70		

Table 2. Results of animal experiments carried out with chromones at Bengers Research Laboratories between 1956 and 1961

Compound	% inhibition histamine release			% inhibition SRS-A release			Activity as antagonists ED ₅₀ µg				% inhibition SRS-A µg/ml	LD ₅₀ i.v. i.p.
	2660 µg/ml	660 µg/ml	320 µg/ml	2660 µg/ml	660 µg/ml	320 µg/ml	Ach	5HT	Nic	Hist		
K18	100	100		91	78			10		11		
BLA 1	60			36	0		> 400	> 500	> 500	1000	2	> 1000
											1000	> 1000
BLA 8	83	55	30	78	62	58				> 100	60	625
										0	1000	> 1000
BLA 13	43	10	0	90	80						0	2500
											100	
BLA 14	55	0	0	78	50	20	> 1000	> 1000	> 100	> 100	0	5500
									0	0	1000	5500
BLA 15	85	25		85	38		> 500	> 500	> 500	> 500		2000
												3500
BLA 18	96	26		100	100	91	124	280	30	160	70	450
											100	
BLA 20	0			81			> 500	> 500	> 500	> 500	0	> 6000
											200	> 6000
BLA 21	45			90			> 500	> 500	> 500	> 500		1500
												> 2000
BLA 25	58	5		100	15					> 100	14	
										0	1000	
BLA 30	55	14		87	100		> 500	> 500	> 500	> 500		750
												750
BLA 50	25			64			> 500	> 500	> 500	> 500		2000
												3500
BLA 68	100	60	30	100	79	40				> 100	41	750
										0	1000	
BLA 80		50	29		45							

against antigen challenge. It was also probably the first chromone to be investigated in the Clinical Immunology Department at the Brompton Hospital by Dr (later Professor) J. Pepys. He reported in 1961 that BLA 8 when injected into the skin significantly inhibited the skin weal response to pollen extract in sensitized subjects at a dose of 10 mg/mL, but not at 1 mg/mL.

Dr W. E. Brocklehurst became a consultant to Bengers and carried out experiments with BLA 8, showing that it both inhibited the release of histamine and SRS-A in guinea pig lung; he also found it was an SRS-A antagonist, but not an antagonist to histamine.

BLA 8 was used by Altounyan in short-term clinical trials in asthmatic patients; the drug was administered orally and he reported a significant increase in basal FEV_{1.0} after 7 days in five out of six patients with proven allergic asthma.

BLA 8 was a gastric irritant but did not appear to have any other toxic effects; its development was discontinued partly because of the project's cessation, but also because it gave only 2 h protection against bronchial antigen challenge.

Human pharmacology

Altounyan had established two important principles by this time. Firstly, that the degree of bronchoconstriction produced by antigen inhalation was related to the dose inhaled and secondly that the degree of protection provided by any drug under investigation was also dependent upon antigen dose.

Figure 10 shows his own dose-response curve to different concentrations of a standard antigen solution (Bencard A12, mixed pollen) each nebulized for 120 s with 8 L/min of air using a Wright nebulizer. This shows a maximum reduction in FEV_{1.0} of >50% when 100% concentration of antigen was used, with a fall of 20% when a 10% concentration was used.

Figure 11 shows the protective effect of compound BLA 8 administered for 5 min as a inhaled 0.5% solution, 10 min before either a 100% or a 10% solution of antigen was administered. With this dose of compound the maximum fall in FEV₁ with 100% antigen is reduced from 54% to 22% (60% protection) and with 10% antigen from 21% to 5% (76% protection).

Altounyan took great care to use the same batch of antigen for as long as possible and when a new batch was needed, to conduct a series of antigen challenges to determine the dose that would produce a maximum fall in FEV₁ of 30–40%. This dose was then used in all subsequent challenges and he checked his own sensitivity to that dose on a regular basis. This is just one example of the meticulous and precise standards he set and why his results could be relied upon. Whilst such practices are standard now they were not in institutions carrying out inhaled antigen challenge institutions at the time.

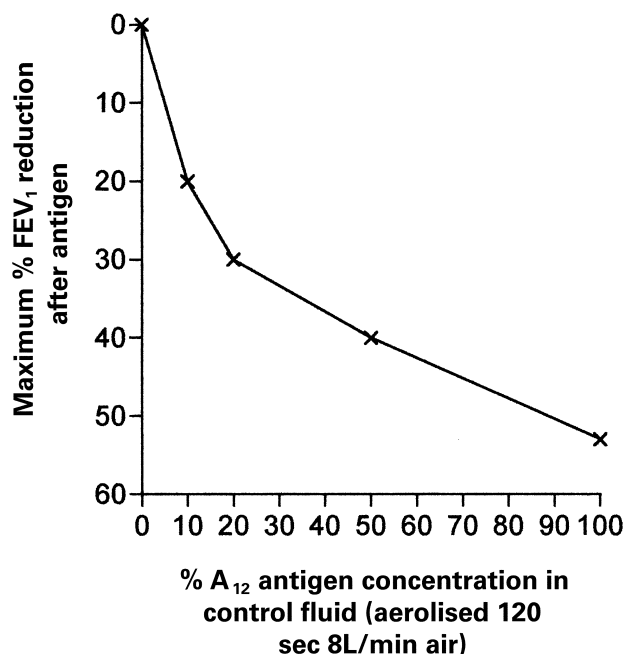


Fig. 10. Maximum percentage fall in FEV₁ after increasing concentrations of inhaled antigen in Dr REC Altounyan (From Altounyan report dated 1961).

The discovery of sodium cromoglycate

The chromone project was not stopped for long – the owners of Bengers Laboratories, Fisons Ltd, removed and replaced most of the Board. A research committee was set up in early 1963 of the main scientists who had remained

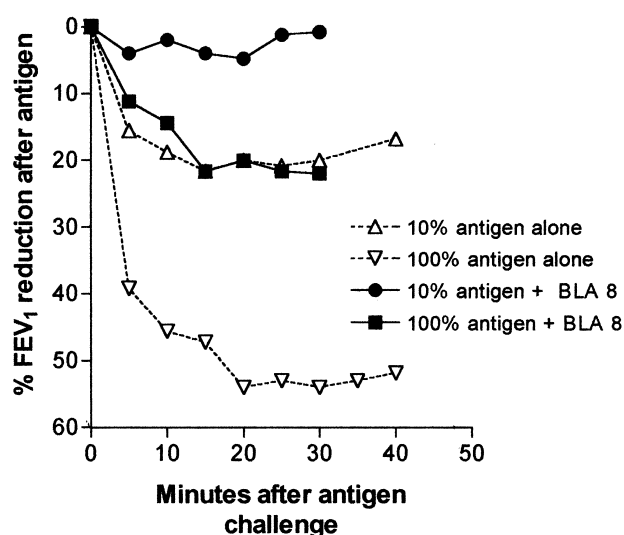


Fig. 11. Percent fall in FEV₁ in after the inhalation of a 10% or a 100% concentration of antigen with and without pretreatment of a 0.5% aqueous solution of BLA 8 inhaled 10 min before antigen.

and it was decided that the only project worth pursuing was the antianaphylaxis (chromone) project — this was therefore reinstated. As many of the previous team had been dispersed, the screening programme for new compounds was initially based entirely on the effects on bronchial antigen challenge in Altounyan himself.

Even though the project had been stopped he had continued to conduct antigen challenges on himself in secret, with the compounds smuggled out of the laboratories to his clinic at Monsall Hospital. The records show that he kept a fairly consistent routine; provided he was well he would carry out bronchial challenges on 3 days a week.

On Mondays and Thursdays, he would carry out a single, standardized antigen challenge and on Tuesdays he would check his nonspecific reactivity using histamine or carbachol challenge, and recorded his FEV₁ with a Palmer spirometer on loan to him by Dr Robinson (Fig. 12).

Any drug tested was administered between 12 noon and 4.00 p.m., with the antigen administered as an aqueous solution using a Wright nebulizer at 4.00 p.m. He then followed through the early reaction to this challenge until

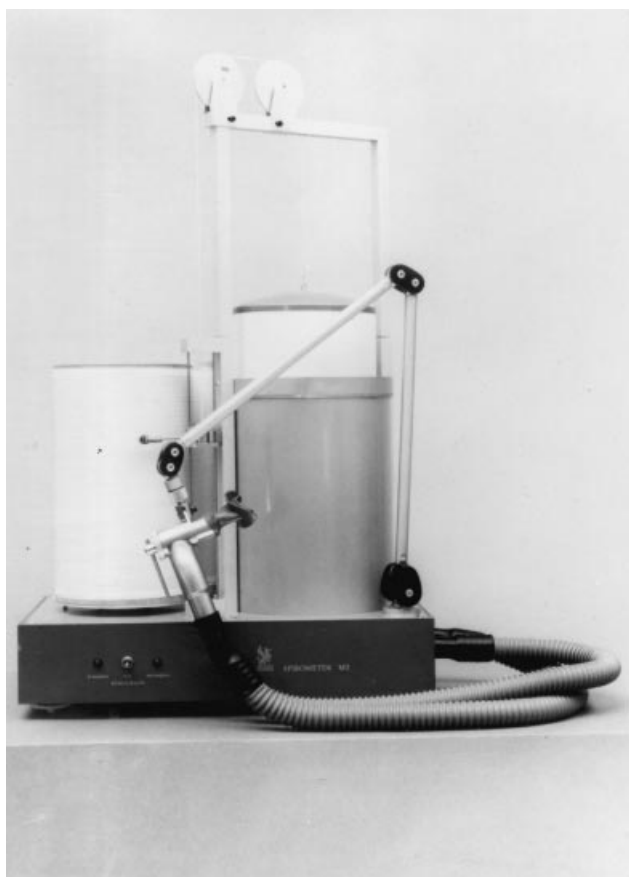


Fig. 12. Direct reading spirometer used by Dr R. E. C. Altounyan in his bronchial challenge experiments.

about 6.30 p.m., when he reversed any remaining bronchoconstriction with inhaled isoprenaline.

Initially he prepared his own antigen by soaking guinea pig hair in water for 4 days and diluting the resulting filtered solution until he arrived at a concentration that gave a consistent immediate bronchoconstriction. This was the inspiration for 'Hair Soup' — the title of the television documentary on his work produced by his daughter, Barbara, though he later used commercial pollen extract allergen from Bencard Ltd.

He set quite strict criteria for a useful anti-asthmatic compound — it would have to give 50% protection for up to 6 h against the immediate asthmatic reaction to bronchial antigen challenge so that on a four times a day dosing schedule substantial 24-h protection would be provided. After rejecting both GR4 and BLA 8 on these grounds the chemists returned to synthesizing further derivatives of khellin.

The first compound that gave the degree of protection Altounyan was seeking was K84 (Fig. 13), which on 9 May 1963 gave 57% protection when administered 1 h before antigen challenge. As can be seen, Altounyan wrote 'Hurrah' on the chart for the first time (Fig. 14), but preceded it with a question mark!

Following these results Roger Altounyan telephoned one of us (JBL Howell) then Senior Lecturer in the Department of Medicine at the University of Manchester and Consultant Physician at Manchester Royal Infirmary, to discuss how the compound might be administered therapeutically in asthmatics. It was agreed that because he had shown it important to inhale the drug prior to the inhalation of antigen, the first trial should be with the drug inhaled continuously for a prolonged period to ensure that the drug was 'in place' before any naturally occurring antigen was inhaled. The drug was therefore nebulized for about 60 h into an oxygen tent containing a volunteer adult male patient with severe, intractable asthma. The result was unequivocal — there was no evidence of any improvement. On retesting himself with the same solution Altounyan found that it was ineffective. Later it was discovered that the remains of the original batch of K84 had been thrown

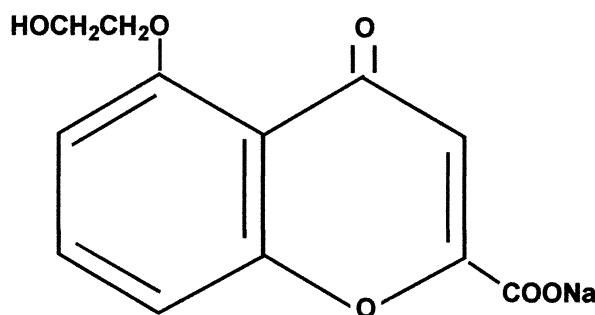


Fig. 13. Chemical structure of K84.

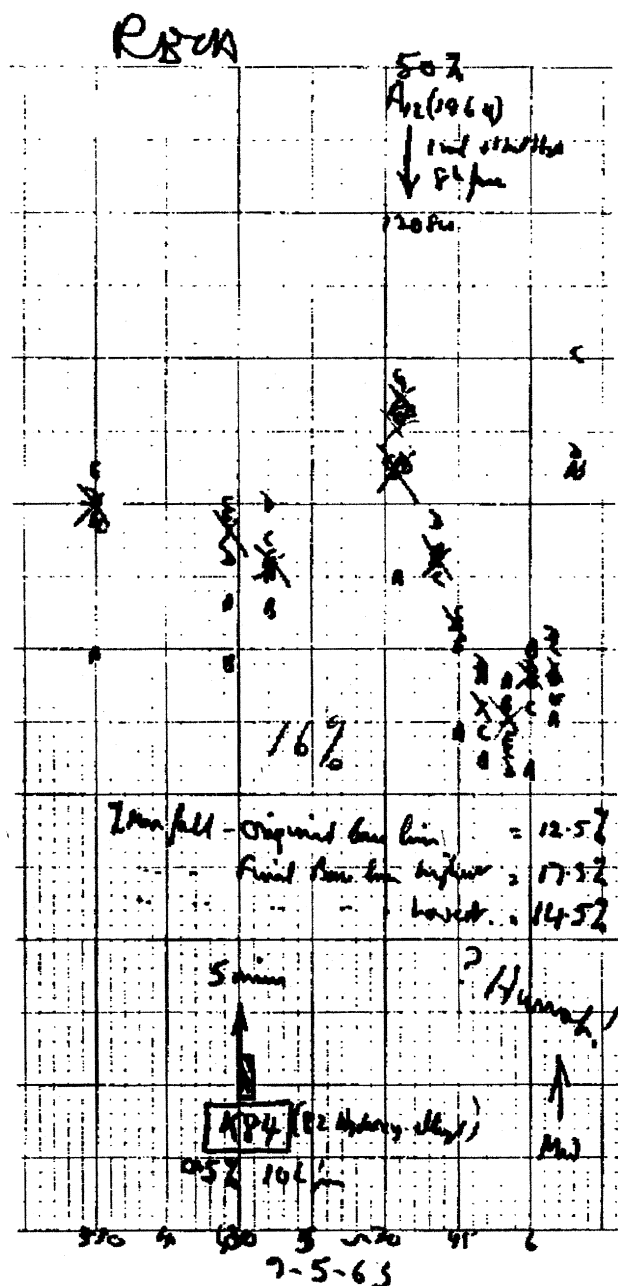


Fig. 14. Chart drawn by Dr R. E. C. Altounyan of bronchial challenge carried out in 1964 showing protective effect provided by K84. Wrote 'Hurrah' on chart for first time.

away and the compound given to the patient and used in the second challenge was from a newly synthesized batch; but no one knew why it was different.

Testing of new compounds continued. Another 18 months were to pass and during a brainstorming session Altounyan suggested that the activity seen with the first batch of K84 might have due to a highly active impurity. The chemical

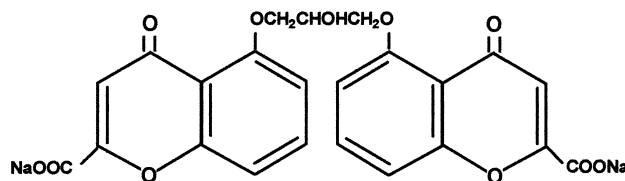


Fig. 15. Chemical structure of sodium cromoglycate.

team at the time, which included Colin Fitzmaurice, John King, Brian Lee and Hugh Cairns, speculated that during the synthesis of K84 two molecules might have joined together and formed a bis-chromone. They then set about to deliberately synthesize some bis-chromones, now referred to as the BL series of compounds.

On 8th October 1964 Altounyan tested the first of these, a bis-chromone from K85, which gave 60% protection. Further compounds in the series followed and the bis-chromone BL670 (later FPL670), sodium cromoglycate (Fig. 15) was synthesized late in 1964.

This was first tested by Altounyan as a 0.5% nebulized solution administered 2 h before antigen challenge on 11 February 1965, when it gave 72% protection.

Further challenge experiments and some patient trials were carried out during the early months of 1965. These were conducted mainly with a pressurized inhaler, which delivered 2 mg per actuation. Altounyan inhaled it himself 4 times a day and also offered it to 6–8 of his severe, steroid-dependent patients. All of them felt some improvement. He gave two inhalers to Howell for trial on two patients, both of whom reported improvement. One of the latter patient's with severe, virtually irreversible airflow obstruction reported rapid symptomatic improvement without any corresponding improvement in FEV₁, which demonstrated from the outset that it could be misleading to rely on objective tests for criteria of benefit. But equally it highlighted the need for a double-blind study of the drug. The experiments that provided the necessary profile of FPL 670 were carried out in June 1965; Altounyan had been ill and his records are not so complete as usual.

His notes for 10 June record

'Sore throat, raw chest. Slight temperature & feeling rotten 6th & 7th and more wheezy. In bed on 7th. Started BL670 20 mg Lomu on evening of 7th, 3x daily. Wheeze much better by 8th although some cough & sputum. Achromycin 8 on 8th, 6 on 9th, 6 on 10th.'

Despite his illness, he carried out an antigen challenge at 4.30 p.m. on 10 June having taken the last 20 mg dose of BL670 at 8 am that morning, 8.5 h before. This challenge resulted in a 23% fall in FEV₁. He started taking BL670 at 20 mg plus isoprenaline, three times daily again.

On 14 June, the last dose was taken 2 h before challenge. The same challenge, a nebulized solution of 10% Bencard A12 antigen inhaled for 120 s at 8 L/min resulted in only a 5% fall in FEV₁.

On 21 June the last dose was taken 6 h before challenge and the fall in FEV₁ was 14% – Altounyan's requirements for more than 50% protection for 6 h had been met.

Altounyan established that the degree and duration of protection against antigen challenge was dose dependent [13] (Fig. 16). In this same report given at the Third International Symposium on Bronchitis (Bronchitis III) held at Groningen in the Netherlands in June 1969, he showed that a single dose, inhaled as a dry powder, had no bronchodilator effect, that it caused a transient fall in FEV₁ similar to that induced by an aerosol of Coca's solution (Fig. 17a), and did not provide protection against challenges by aerosols of histamine, bradykinin, serotonin or carbochol. In contrast when the drug was inhaled 1 min or 1 h before an antigen it markedly inhibited the response (Fig. 17b). It was progressively less effective when administered 1, 6 and 15 min after the antigen (Fig. 17c). To satisfy the requirement that adequate protection should be maintained for up to 6 h, it was necessary to increase the dose to 20 mg. A 20-mg dose could not be delivered from a metered dose aerosol, so

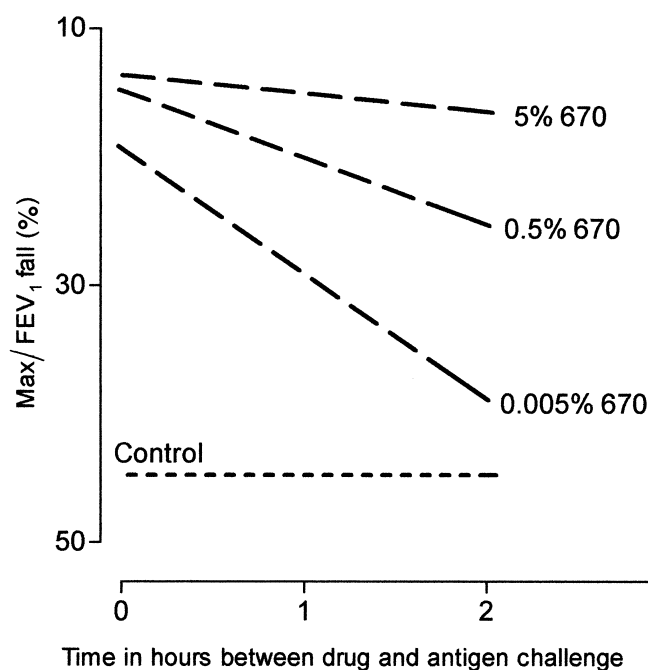


Fig. 16. Maximum FEV₁ reduction after antigen alone and after antigen and varying concentrations of DSCG inhaled 5 min or 2 h before challenge. Reproduced from Altounyan REC. Disodium cromoglycate – clinical pharmacology in obstructive airways disease. Bronchitis III. Royal VanGorcum, Asses The Netherlands, 1970.

a Lomulizer dry powder delivery system, developed by Bengers, initially was used. This was soon replaced by a modified version of the dry powder delivery system invented and developed by Altounyan, the Spinhaler®. This has remained in use to this day.

The transient bronchoconstriction occurs as BL670 is a salt. The inhalation of 20 mg resulted in a 4% fall in FEV₁ for Altounyan, but this can be greater in some patients. In

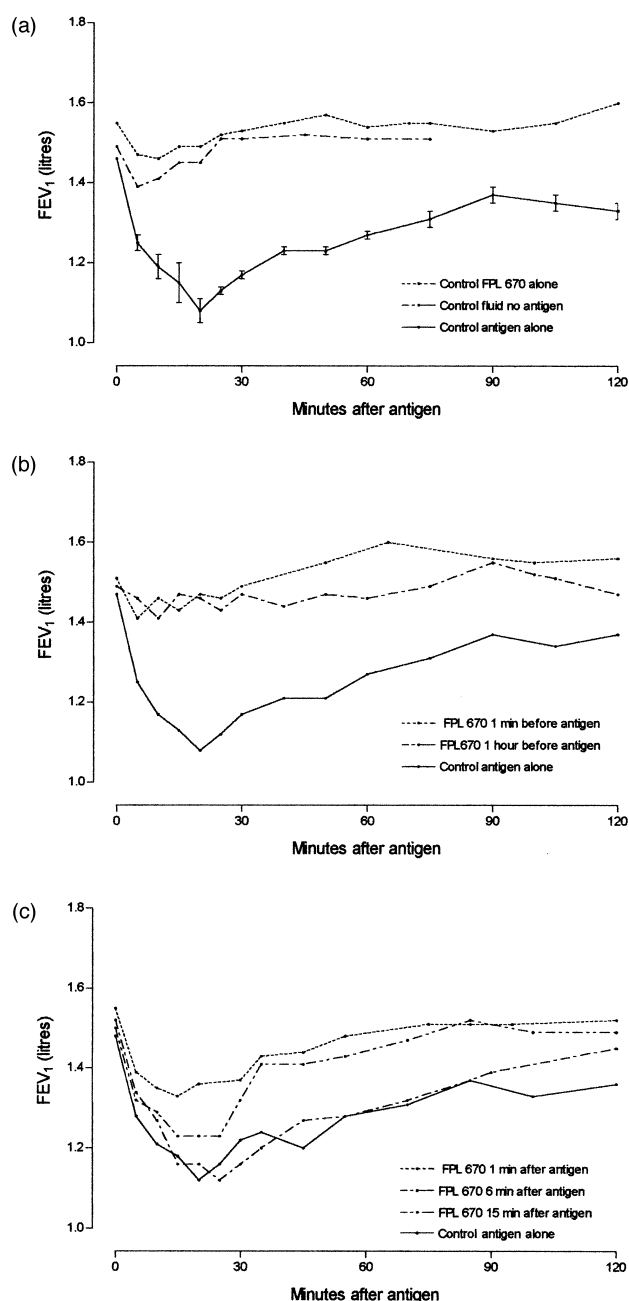


Fig. 17. Challenge studies carried out by Dr R. E. C. Altounyan with FPL 670 (sodium cromoglycate).

order to prevent this Altounyan added a small amount of isoprenaline, a short acting bronchodilator to the first product to be launched, Intal compound, which contained 20 mg of sodium cromoglycate, 0.1 mg of isoprenaline and 20 mg of lactose.

Sodium cromoglycate

Sodium cromoglycate is an extremely potent drug; in his first publication on its main characteristics [14], Altounyan reported:

'The maximum protection (70%) occurred at an estimated dose of 1.0 mg inhaled 10 min before challenge but some protection (40%) was observed at a dose as low as 0.15 μ g. When the time interval between drug inhalations and antigen challenge was extended to 2 h, no protection was observed with the lower dose, although some protection was still evident following a dose of 1.0 mg. A single clinical dose (20 mg) inhaled as a micronized powder from a specially designed appliance afforded greater than 50% protection for at least 6 h.'

The first randomized, double-blind, clinical trial with inhaled sodium cromoglycate was carried out by Howell and Altounyan. This was considered to be pivotal both to establish the efficacy of the drug and to convince the Board of Bengers that the development should continue. Because of the very limited testing for safety which had been carried out it seemed ethical for the first double-blind trial to study the smallest number needed of the most severe patients requiring worryingly large doses of oral corticosteroids. Ten such patients were chosen for a double-blind crossover trial using sequential analysis and this design meant that it was necessary to decide in advance the way in which benefit, if it occurred would be judged. Because of the experience with the patient who had demonstrated that lung function tests could be poorly correlated with improvement, it was decided that the only sensible way to judge the benefit was for the clinician, taking all the clinical evidence into account but not including the results of lung function tests, to come to a clinical judgement. Clinical evidence included daily record cards kept by the patient. Lung function tests were measured but only revealed after the clinical decision had been made. The mean age of the patients was 45.7 years, and their mean daily dose of oral prednisolone was 15.25 mg (range, 10–20). Their mean FEV₁ at the end of the initial control period was 0.96 L (range, 0.43–2.06 L). The results were unequivocal and it is worthwhile recording the key section from the publication in the *Lancet* [15] — 'When 10 patients had completed the trial the first analysis was made. It was then found that all the preferences were in favour of FPL670 and it will be seen from the sequential analysis graph (Fig. 18) that the sample path crossed the upper boundary at the seventh preference. The exact probability

of this result, being due to chance, is 0.016. The trial was therefore terminated'.

Although the change in FEV₁ was not used in judging benefit, it is worth noting that the mean increase in FEV₁ after isoprenaline was 0.01 L and after FPL670 + isoprenaline was 0.29 L. This difference is significant, $P = 0.0019$.

This trial was conducted during September and October of 1965 but publication of the results was delayed until September 1967, because of the long delay before the drug became available.

These were early days of the Committee on Safety of Drugs in the United Kingdom, the government agency set up to approve marketing applications of new drugs. Howell and Altounyan also carried out the definitive trial to support the marketing application.

Many patients at Monsall Hospital and later, the Manchester Royal Infirmary were offered therapy with FPL670 from April 1965, initially with a metered dose aerosol delivering 2.0 mg of sodium cromoglycate and 0.1 mg of isoprenaline per dose. They were transferred to the 20 mg sodium cromoglycate + 0.1 mg isoprenaline as supplies of the dry powder capsules and Spinhalers became available later that year. Case no. 1 in the report is Altounyan himself who is described as 'an atopic subject with symptoms of mild to moderately severe perennial asthma.' The details of the clinical effects in the first 100 patients was published in 1969 [16]. The first scientific paper on sodium cromoglycate was published by the then Director of Research and Development at Fisons, Dr J. S. G. Cox in 'Nature' in 1967 [17].

The clinical section of the marketing approval dossier was signed by Altounyan and Howell on 9 September 1966,

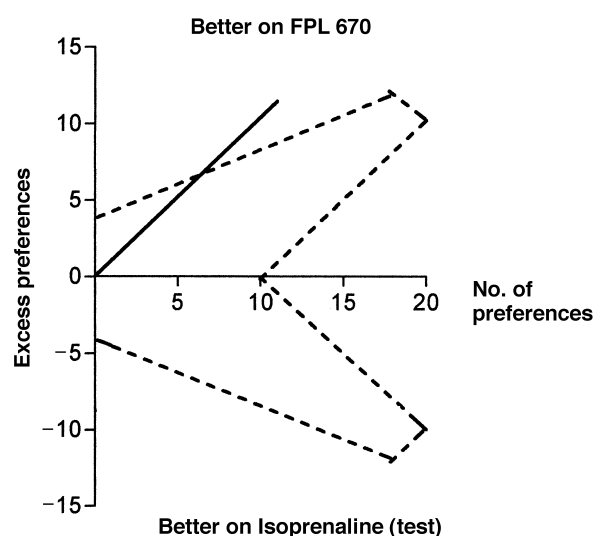


Fig. 18. Sequential analysis of results of trial of FPL670 and isoprenaline. Reproduced with permission [15].

approval was granted in 1967 and Intal Compound was launched in the United Kingdom in January 1968.

The first major symposium at which a number of clinicians and scientists described their work with sodium cromoglycate was held at the Royal Society of Medicine in London in 1969, under the chairmanship of Professor J. G. Scadding and Dr A. W. Frankland – a report of the proceedings was edited by Professor J. Pepys and Dr Frankland [18].

Bronchial challenges carried out by Altounyan on himself

Altounyan kept most of the records of the bronchial challenges he carried out on himself since the first in July 1957. From then until he first tried sodium cromoglycate in February 1965 his records show that he carried out challenges on 615 days, testing 206 different compounds, details of which are presented in Table 3.

The majority of these challenges were antigen challenges which were either antigen alone, or an antigen preceded by a chemical supplied by the Research Department.

In the early days these had been screened and subjected to a limited amount of safety testing, but after the project was stopped in 1961, this did not happen and the compounds were supplied directly to Altounyan from the Chemistry Department.

When the project was reinstated in 1963 no preliminary animal screening was carried out and only limited animal toxicity testing.

Altounyan's notes record that some of the compounds were extremely bitter and irritant, leaving him with quite severe side effects. As well as establishing the basic

principles of antigen challenge testing, Altounyan also investigated histamine and carbachol challenges. Initially he used the histamine challenges to test a series of new Bengers antihistamines and used both challenges to test the efficacy of new formulations of isoprenaline and isoprenaline plus atropine-metered dose inhalers.

Altounyan considered that his work investigating the relative bronchodilating properties of isoprenaline and atropine was important in discriminating between the mechanisms of chronic airways obstruction and the phases of allergic asthma. This was published in a paper in 'Thorax' in 1964 which also included details of the challenges he had carried out on himself while investigating the protective effects of atropine, isoprenaline and mepyramine against carbachol, histamine and antigen challenge [19].

Sodium cromoglycate today

Inhaled sodium cromoglycate remains one of the important preventive treatments for asthma. In retrospect it is perhaps unfortunate that under pressure from 'opinion leaders', it was decided to introduce a plain version of Intal, without added isoprenaline.

Altounyan's original specification for Intal Compound – 20 mg sodium cromoglycate, 0.1 mg isoprenaline with 14.9 mg of lactose as a carrying agent probably remains the optimal of the sodium cromoglycate products in asthma management.

The definitive clinical trial in 1972 by the Medical Research Council of the United Kingdom [20] was conducted in adult patients with severe asthma; it reported that after 12 months treatment, 80% of the patients treated with sodium cromoglycate plus isoprenaline remained well controlled, compared to 67% on sodium cromoglycate alone, 25% on isoprenaline alone and 16% on placebo.

This was supported by a trial carried out in Belgium, Germany and France [21], which found that 57% improved with sodium cromoglycate alone compared to 80% on sodium cromoglycate plus isoprenaline.

More recently a trial using nebulized aqueous solutions in children with moderate to severe asthma carried out in Japan [22], compared the combination of 20 mg of sodium cromoglycate and 1 mg of salbutamol with salbutamol alone and with sodium cromoglycate alone. This showed an overall improvement in asthma in 71–90% of those treated with the combination compared to 58–64% with salbutamol alone and 58–62% with sodium cromoglycate alone.

These results suggest a possible synergistic effect between sodium cromoglycate and Beta₂ agonists. It is also likely that the addition of a bronchodilator both increases the dose to the lung [23], and the evenness of distribution of the drug in the bronchial tree which has been shown to be important in terms of efficacy [24].

Table 3. Documented bronchial challenges carried out by Dr R. E. C. Altounyan between July 1957 and February 1965

Year	Number of days when recorded challenges were carried out	Number of chemical compounds tested
1957	5	2
1958	28	5
1959	42	8
1960	74	35
1961	106	65
1962	136	31
1963	68	14
1964	138	37
1965 (up to 11/2/65)	18	9
Total	615	206

Intravenous use of sodium cromoglycate

The first evidence that the distribution of the drug on the bronchial mucosa that determines clinical efficacy, rather than the total delivered dose, was provided by Altounyan.

During the development of the 1.0 mg-metered dose inhaler in 1977, he carried out a series of bronchial antigen challenges on himself whilst he was receiving an intravenous infusion of sodium cromoglycate [25]. The challenge was performed after 30 min, when it was calculated that a steady blood level had been reached. The maximum estimated blood level attained was 500 ng/mL, with optimal protection achieved at 200 ng/mL. However, the optimal protection at 20% was far lower than that given by inhaled doses that have subsequently been shown to give peak plasma levels of 46 ± 7 ng/mL after the inhalation of a 20-mg capsule. Blood levels would appear to be unrelated to clinical activity.

Chromones after sodium cromoglycate

The story of Altounyan and the chromones does not end there; he continued to carry out bronchial challenges on himself up until ill-health forced his retirement in 1985.

Despite the development of numerous animal and *in vitro* models of asthma developed since the introduction of sodium cromoglycate, no tests proved to be of any better predictive value than the ability of drugs to inhibit the effects of bronchial antigen challenge in man.

Following the successful introduction of sodium cromoglycate, the first task to be given to the new Research and Development Group at Fisons Pharmaceuticals was to develop an orally active chromone. The first of these was FPL52757 (Fig. 19), which entered Phase II clinical trials in 1975. These trials were stopped fairly rapidly when a significant number of patients developed raised liver enzymes. This was a major setback for the team as this compound had gone through chronic toxicological testing without any problems in five animal species, including two primate species — an example of the problems and potential dangers of trying to extrapolate from animal experiments to man. Development work on the second compound, FPL57787 (proxicromil) followed soon after. (Fig. 19). Although this compound was supposed to have great potential, Altounyan's own experiments, which he reported at a 1977 symposium [25], showed that even after optimal dosing it gave only about 40% protection against antigen challenge 4 h after the last dose. It failed to show any benefit in clinical trials in asthma, was found to be potentially carcinogenic in rats and its development was stopped.

Altounyan continued to screen chromones throughout the 1970s — at the end of the 1970s when his worsening chest problems made the winters in the UK difficult, he spent the

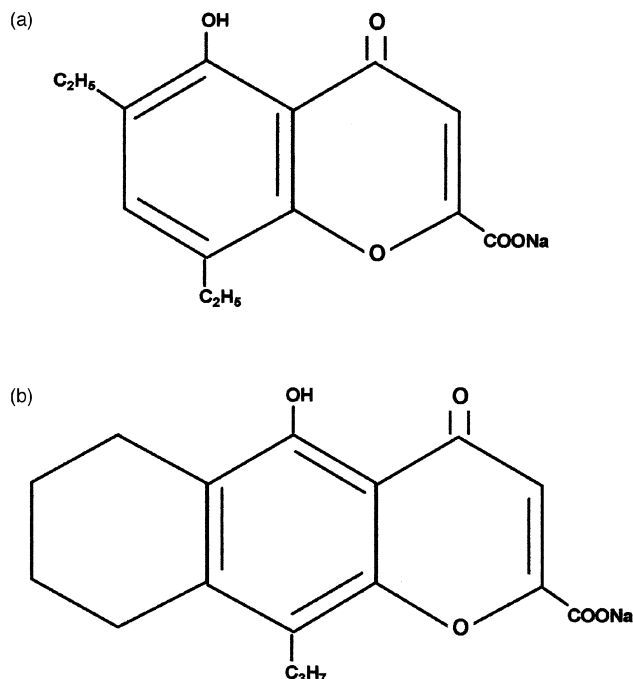


Fig. 19. Chemical structures of oral chromones, (a) FPL55757 and (b) FPL55787.

winter months in Sydney, Australia, where he was a visiting clinical pharmacologist in the Department of Thoracic Medicine in the Royal Prince Alfred Hospital in Camperdown, New South Wales. This period produced the first description of the relationship between saline tonicity of inhaled solutions and the degree of bronchoconstriction produced in asthma patients [26]. At this time Altounyan was supposed to have been stopped from testing the new drugs on himself, but compounds were still being sent out to him.

In a telex from Australia to the R & D Director in the UK dated 26 March 1980, he reported:

'At 6 h 360 gave 64% protection, 002 gave 33%, 670 gave 53%. Poor atropine response may have caused relatively high value for 670. In terms of lowest FEV value after antigen 360 looks even more effective. I plan to study next 392 then 59787 at 2 h after — I would welcome your suggestions. I leave Wallaby on 11 April regards Roger.'

Decoding his abbreviations; 360 was FPL59360KP (Fig. 20) or minocromil sodium, 002 was FPL59002KP or nedocromil sodium (Fig. 20) and 670 was still FPL 670, sodium cromoglycate. In retrospect one might ask why the next chromone to be developed was nedocromil rather than minocromil but that is another story.

In therapeutic trials nedocromil sodium has been shown to be an effective treatment for asthma [27], with many animal and *in vitro* results demonstrating its superior potency over sodium cromoglycate.

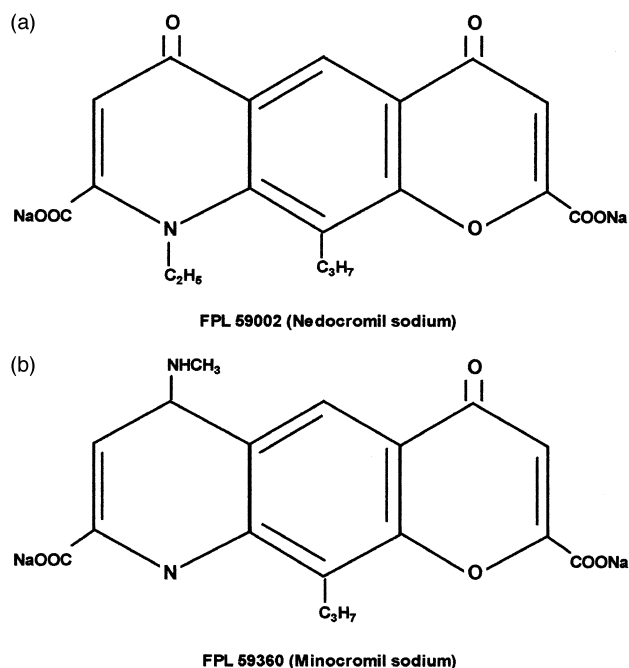


Fig. 20. Chemical structures of new inhaled chromones, (a) FPL 59002 (nedocromil sodium) and (b) FPL 59360 (minocromil sodium).

However in the two therapeutic trials comparing the two compounds which can be regarded as being adequate from a design point of view, the two drugs are essentially equivalent in efficacy. Differences that are seen in the analysis are in favour of sodium cromoglycate in the one trial [28] and in favour of nedocromil sodium in the other [29]. In both trials the dose of sodium cromoglycate was 8 mg/day and nedocromil sodium 16 mg/day.

Altounyan himself compared the efficacy of the two compounds in a series of challenge studies carried out in healthy, atopic volunteers [30]. In these he showed the two drugs provided equal protection against antigen and 'fog' challenge but that nedocromil sodium provided better protection against sulphur dioxide challenge (Fig. 21).

It was also in this paper that Altounyan described his last, well almost, last invention. As he began to use healthy atopic volunteers in challenge tests he considered that subjecting them to repeated falls of 20–30% in FEV₁ was not acceptable.

Together with his son Peter and with his long serving nurse technician Margery Cole, he developed a computerized system, which measures flow at any preselected lung volume on the expiratory flow volume curve (The Collingwood Measurement). This is achieved with a single breathing manoeuvre. They found that measuring the flow rate at a standard lung volume (TLC less 70% of VC) is 4–8 times more sensitive as an index of bronchoconstriction than

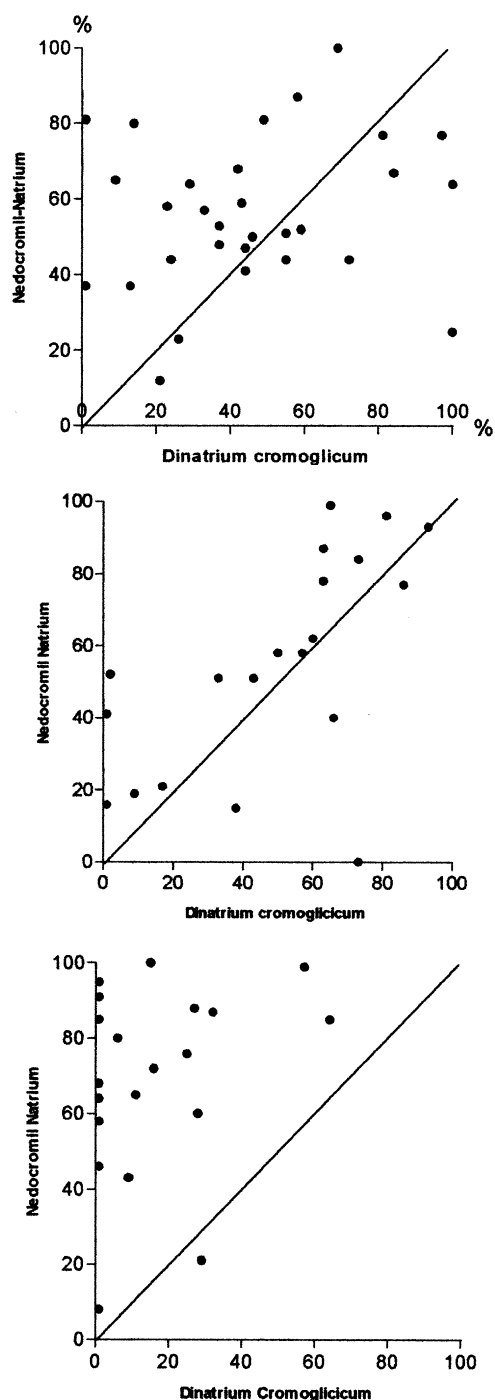


Fig. 21. Results of bronchial challenge experiments carried out by Dr R. E. C. Altounyan showing comparisons of protective effects of nedocromil sodium and sodium cromoglycate against antigen, fog and sulphur dioxide challenge. Reproduced with permission from [30].

FEV₁. Changes of greater than 50% in this measure equated to changes in FEV₁ of less than 7% and were thus undetectable to the subject being tested; this also meant that the severity of the challenge could be reduced.

Sodium cromoglycate today

Inhaled sodium cromoglycate (Intal[®], Lomudal[®]) was introduced at a time when there had been no new treatment for asthma for many years. It rapidly became widely used in asthma and the company then responsible — Fisons Pharmaceuticals — who were not known for pharmaceutical products at the time, were extremely successful in securing its early introduction in most of the major countries across the world.

Indeed, sodium cromoglycate became one of the most successful drugs introduced into clinical medicine and was soon recognized as providing one of the best balances between efficacy and safety of any drug used in the treatment of asthma.

Apart from the initial bronchoconstriction that can occur in some patients, no serious side effects or long-term toxicity problems have been reported. This excellent safety profile is primarily a consequence of its pharmacological activity being confined to the mucous membrane to which it is applied; and any sodium cromoglycate which is systemically absorbed is rapidly eliminated in an unchanged, non-metabolized state.

Two major development breakthroughs were required before sodium cromoglycate could be exploited commercially. One was the invention of a continuous reactor by one of the chemists, Colin Fitzmaurice, which made the rather cumbersome synthesis of the chemical a lot easier and the other was the invention of the Spinhaler by Roger Altounyan.

However the drug was introduced when the pharmaceutical industry was growing rapidly and becoming extremely competitive. 'Intal' was seen as a great threat to the new β_2 agonists, the inhaled corticosteroids and in the United States the sustained release theophyllines, all of which were introduced at about the same time.

This competitive element led to a situation where the relative usefulness of all the available drugs may not have been optimized. Many of treatment guidelines that have been introduced in recent years see the inhaled chromones and the inhaled corticosteroids as 'either/or' rather than two different types of anti-inflammatory agent which can work synergistically.

We suggest that the summary of the relative position of the two types of agent described in the early paper by Altounyan and Howell (1969) still applies:

'In view of the apparently similar clinical effects of disodium cromoglycate and corticosteroids, it is tempting to try and equate the effectiveness of these two forms of therapy. But this is not possible as the therapeutic effects

of disodium cromoglycate and corticosteroids are not wholly interchangeable. For example, in those patients on maintenance corticosteroid therapy, a minimum dose of corticosteroids is usually necessary, below which disodium cromoglycate is without clinical effect. On the other hand we have observed patients in whom corticosteroids alone or even in high dosage could not achieve the same therapeutic response obtained by a combination of a lower dose of corticosteroids together with sodium cromoglycate. From these observations of the effects of sodium cromoglycate and corticosteroids, it appears that at least two processes may co-exist in asthma, one being mainly responsive to sodium cromoglycate and the other to corticosteroids. The fact that in some subjects sodium cromoglycate may partially or completely replace corticosteroids or vice versa suggests that these two processes overlap to a varying degree. The difference between these processes may lie in the immunological mechanisms concerned or in their anatomical location.'

This was written over 30 years ago and referred to orally administered corticosteroids. If one was to substitute inhaled corticosteroids, we believe the same would apply today.

Chromones are traditionally classified as 'mast cell stabilisers' as it is believed that their primary mode of action is to inhibit the release of mediators of inflammation from mast cells. Whilst this effect on mast cells is undoubtedly correct and is an important preventive attribute, it is less certain that this effect is important for their longer-term beneficial effects in asthma.

Altounyan would have been intrigued to learn of one the most recent *in vitro* findings relating to the chromones — that they can inhibit the synthesis of IgE antibodies by human B lymphocytes. This was first reported by Kimata in Japan in 1990 [31] and confirmed by Loh *et al.* in the USA in 1994 [32]. What is interesting about these findings is that they fit more closely with the expected clinical efficacy of the chromones than do other models. In their experiments using B lymphocytes from healthy human donors, Loh *et al.* reported that at a dose of 1 μ M sodium cromoglycate there was virtually complete inhibition of IgE synthesis by IL-4-stimulated purified B cells in 11 out of 17 subjects tested (65%) and inhibition of <50% in the remaining six subjects (35%).

In a 4-week therapeutic study in which bronchoalveolar lavages were performed before and after treatment [33], using the improvement in symptom score as the basis of benefit, Diaz *et al.* defined 14 clinical responders (74%) and five nonresponders (26%). She also carried out bronchoalveolar lavages before and after treatment and showed there was a significant reduction in house dust mite-specific IgE antibodies in the lavage fluid and a nonsignificant increase in the nonresponders, with no change in those treated with placebo.



Fig. 22. Roger Altounyan sailing 'Mavis' (Swallow) on Lake Coniston.



Fig. 23. Roger Altounyan at dinner at his 65th birthday party at which he insisted on smoking his pipe whilst taking intranasal oxygen.

These results may indicate that at a local surface level the interference of the chromones in local IgE synthesis may be of greater importance than the effect on mast cells and may account for their longer term benefits.

Perhaps 'Intal', the name given to the first inhaled product of sodium cromoglycate because it 'Interferes with Allergy' was closer to the truth than was then realized!

The contribution of R. E. C. Altounyan

Altounyan died in December 1987. This review has concentrated on his work up to and including the discovery of sodium cromoglycate and has in the main ignored all the additional compounds he tested on himself during the 1970s and early 1980s. It is hoped that this review will allow his contribution to the treatment of and understanding of the nature of asthma to be further appreciated.

At a birthday party in the Lake District with some of his medical contemporaries in October 1987 to celebrate his 65th birthday, he outdid them all by remaining longest on deck of a boat trip along one of his much-loved Cumbrian lakes, Coniston, on a cold and wet October day (Fig. 22).

At dinner that night he demonstrated his last invention, a balloon inserted into his oxygen supply line which he could squeeze manually to give an oxygen boost to allow him to move out of the wheel chair to which he was then confined. He also succeeded in frightening the dinner party by insisting on lighting his pipe whilst inhaling intranasal oxygen! (Fig. 23).

Perhaps the last word on the mode of action of the chromones should be from him. At the review of the mode of action of sodium cromoglycate he presented at the symposium in Davos in 1979 he concluded his talk with:

'Tell me first what asthma really is, then perhaps I could tell you what cromoglycate does to it!'

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