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C ACTINOMYCIN ACTION IN ANAPHYLAXIS AND ALLERGY

By

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Studies on antibiotics have demonstrated that the depressing action on the biological activity occurs not only on micro-organisms' cells, but also on tissue cells. An evident cytostatic action has been largely observed for Actinomycin. Various types of this antibiotic have been recognized; A Actinomycin by Waksman and Woodruff, B Actinomycin by Lehr and Berger, X Actinomycin by Linge are similar types and have been isolated from *Streptomyces antibioticus*, or like species. Another type is J₁ Actinomycin by Hirato and Nakanishi, isolated from *Streptomyces flavus*. The existence of other similar antibiotic substances is assumed.

A new Actinomycete, *Streptomyces Chrysomallus*, was revealed and identified in 1946 by v. Plotho and Lindenbein; from this actinomycete, Brockmann and Grubhofer isolated, in crystalline state, an antibiotic substance showing a considerable activity which was named C Actinomycin. By later investigations Actinomycin C was found to embrace three different antibiotic types, C₁, C₂ and C₃. In C Actinomycin small amounts of C₁ are present, while C₂ and C₃ are found in equal proportion. Brockmann considers Actinomycin as a natural staining substance and suggests to name it chromo-peptide.

The researches carried out in the Bayer Scientific Laboratories pointed out some biological properties of C Actinomycin (Sanamicina). An evident lymphotropic action of this

antibiotic has been particularly proved: animals (mice) treated with Actinomycin showed, in comparison with the controls, a very marked diminution of the average weight of the spleen. While in the control animals the spleen average weight of the spleen was 115 mg, in those injected with 30 μ g of C Actinomycin in 3 days the average weight of the spleen had been reduced to 34.5 mg. The histological examination showed that the atrophy of the spleen depended on a reduction of the lymphoid component. The lesion of the lymphoid tissue seems to be reversible. Other histological investigations (Hackmann, etc.) on mitoses in Galeati's crypts showed that the cytostatic action of C Actinomycin sets in especially during mitosis, with great harm to the nuclear substance.

This cytostatic, particularly lymphotropic action demonstrated by the first researches by Domagk & co-workers and, later on, by clinical experiences (Schulte) demonstrating a particular therapeutic action on lymphogranuloma, led us to examine the C Actinomycin activity on anaphylactic phenomena and allergic syndromes in general. The correlations between immunity and the reticulo-endothelial system or, more particularly, the lymphatic system, have been amply proved. A large number of researches, carried out from various points of view, could demonstrate the importance of the lymphatic tissue in the antibody-formation. An evident participation of lymphatic structures, and notably of the less evolved elements, i.e. histiocytes and lymphoblasts, during hyperergic reactions has been histologically demonstrated. These elements assume clearer aspect, and modify their relations with the other tissue cells. Since 1929 Epstein had found a connection between immunitary conditions and formation of nodules in which he distinguished reticular and lymphoplasmocytary cells. At the present time the formation of antibody gamma-globulins is ascribed by some authors to lymphocytes, and by others to plasmocytes. Keuning has recently suggested the great importance of immature cells of the lymphoid tissues in this antibody-genetic function.

Without entering into analytical details of the importance of the various cytological components, the noticeable function of the lymphoid tissue in the antibody-genesis lead us to consider the possibility of interfering in hyperergic reactive phenomena by examining the lymphoid tissue. Various attempts have been made on this subject.

Bis-beta-chloro-ethyl-amine derivatives, known as nitrogen mustards, chloramines or azo-hyprites, exert on tissues various actions proved by us in previous histological examinations. For instance, a change of the lymphoid tissue with reduction of the follicular areas, damage of the germinative centers and histiocytary lesions (nuclear dilatation and fragmentation) was observed in the spleen. Similar alterations have been observed also in the lympho-nodes. Nitrogen mustards have been demonstrated to have an anti-anaphylactic and anti-allergic action (Jimenez Diaz and co-workers, Corelli, Businco, etc.).

Cortisone, too, has a well known lymphotropic action. Histological examinations carried out by us showed that cortisone does not possess, as generally assumed, a lymphocytolytic action, but rather exerts a damaging effect on the immature histiocytary elements of the germinative centers and particularly on the cells devoted to the lymphoblastic activity. However, the numerous experiences do not show cortisone to be able to protect animals from anaphylactic shock, while a more evident protective activity is observed against cutaneous hyperergic manifestations. On the contrary, a very clear cortisone action, though restrained to the symptomatological phenomena, has been demonstrated towards allergic syndromes (asthma, etc.).

Both the up to-date experimental researches and the clinical investigations have demonstrated—as above mentioned—an important lymphotropic action of C actinomycin, resulting in significant cytostatic phenomena in the tissues. A study taking into consideration the possibility of this antibiotic acting both on anaphylactic and allergic manifestations appeared to be “a priori” interesting.

RESEARCHES ON ANAPHYLACTIC SHOCK

Experiments have been carried out on guinea pigs of the anaphylactic shock resulting from the action of C Actinomycin (Bayer Sanamicina). The following procedure has been employed: A group of control animals (A) were sensitized with egg albumin; after a suitable interval a reacting injection was given. A second group (B) after sensitization with egg albumin, received, every two days, and throughout the sensitization period, intracardiac injections of 2 μ g Actinomycin dissolved in a physiologic saline solution. The same treatment was employed in a third group (C) with a higher dose of Actinomycin (10 μ g dissolved in half ml of physiologic saline solution). The sensitized animals of the fourth group (D) received an intracardiac injection of 2 μ g Actinomycin half an hour before the reacting injection. In a fifth group, the same procedure was followed, with a 10 μ g dose of Actinomycin.

Aim of the present research is a comparative investigation on the action of various doses of Actinomycin and the study of a possible difference of behaviour between the prolonged treatment and that restrained only to the reacting phase.

RESULTS

Group A (controls).—The reacting injection, administered 21 days after the first injection, induced severe and rapid anaphylactic shock in all animals accompanied by itching, convulsions and followed by death in an average space of time of 3 minutes. 100 per cent's mortality.

Group B (guinea-pigs sensitized and treated with 2 μ g of Actinomycin every 2 days). The reacting injection, administered 21 days after the first injection, induced conditions of severe suffering followed by death from anaphylactic shock in an average time of 3'50". 100 per cent's mortality.

Group C (guinea-pigs sensitized and treated with 10 μ g of Actinomycin every two days). The reacting injection administered 21 days later demonstrated in all animals crises of

anaphylactic shock followed by death in an average time of 3'30". 100 per cent's mortality.

Group D (guinea-pigs sensitized and injected with a 2 μ g dose of Actinomycin half an hour before the reacting injection). The reacting injection, administered also in this group 21 days after the sensitizing injection, induced severe conditions of suffering, not completely typical of anaphylaxis, with alternated phases of torpidity and agitation, followed by death in an average time of 4'30". 100 per cent's mortality.

Group E (guinea-pigs sensitized and injected with a 10 μ g dose of Actinomycin half an hour before the reacting injection). The reacting injection, administered 21 days after the sensitizing injection, induced severe but not typical anaphylactic conditions in all animals, followed by death in an average time of 5'10"; 1 animal only survived the crisis. 90 per cent's mortality.

From the registered experimental data, and according to our technical procedure, it may be concluded that Actinomycin does not protect against lethal anaphylactic shock from albumin egg. However, in animals treated with Actinomycin the period of survival is more prolonged than in the controls. Such a period of survival seems to be related to the dose employed and, according to our experience, it is more marked when Actinomycin injection is practised half an hour before the reacting injection.

From other researches carried out by us (Businco, Crifò, Connestabile, Ferrari) on guinea-pigs, the following comments on C Actinomycin action have been made:

General conditions.—A sufficient tolerance to the employed doses of the drug has generally been observed. No particular reaction was observed immediately after injection and later. Good appetite and normal vivacity were usually noted.

Mortality.—Death occurred at the first injections. In the group receiving doses of 2 μ g, the mortality has been of the 10 per cent; in the animals receiving 10 μ g of Sanamicina it reached the 15 per cent.

Weight.—A loss of weight was observed during the first week of C Actinomycin therapy; later on such loss was reduced and in some cases even compensated by a ponderal increase, as if an adaptation towards the antibiotic had occurred in the animals.

Hair system.—An interesting observation is given by behaviour of the hair system. Animals treated with 2 μ g, and particularly those treated with 10 μ g showed a marked lability of the hair system. A considerable loss of hair was in fact observed in the animals only by seizing them; and a fall of large tufts was easily caused by drawing out hairs.

Hematological picture.—As regards red and white blood cells, no significant variations in number were induced by Actinomycin. Qualitatively, a certain increase of eosinophil leukocytes seemed to occur after the first intracardiac injections of Sanamicina. At the end of the experiment a return to normal values was observed. As regards lymphocytes, an evident increase in number has been found in a majority of cases.

Plasma-proteins.—A preliminar investigation on plasma-proteins has been carried out in a group of normal guinea-pigs. The average values obtained are the following: total proteins 7.49; albumins 3.94; total globulins 3.55; α_1 and α_2 globulins 1.70; beta globulins 0.90; gamma globulins 0.95; albumin-globulin ratio 1.11.

The same investigation has been undertaken on a group of guinea-pigs previously treated with 9 intracardiac injections of Sanamicina in a single dose of 10 μ g. The average values obtained are the following: total proteins 4.98; albumins 3; total globulins 1.98; α_1 and α_2 globulins 1.41; beta globulins 0.30; gamma globulins 0.27; albumin-globulin ratio 1.50.

From a comparative examination of the protein values, before and after C Actinomycin therapy, it may be deduced that the antibiotic induces a total hypoproteinemia chiefly affecting globulins, and gamma globulins in particular.

Autoptical findings.—Post-mortem examinations, carried

out on animals that died during C Actinomycin treatment, or were killed at the end of the experiment, showed a congested condition of varying degree and in some cases the presence of a hemorrhagic focus (digestive tract, etc.). But the most interesting finding was represented by a very conspicuous dilatation of cholecyst, sometimes reaching the volume of a small olive clearly visible on the surface of the liver. The cholecyst was filled with biliary fluid, and its internal surface showed signs of hyperemia and phlogosis.

RESEARCHES ON ALLERGIC DISEASES

The registered experimental researches were followed by the study of C Actinomycin action in human allergy. We have studied, till now, a group of 15 patients: 7 were suffering from bronchial asthma, 7 from urticaria and 1 from chronic colitis. All patients have been treated with intrav. injections of Bayer Sanamicina. The treatment commenced with a daily dose of 40 μ g, and then gradually increased. The daily dose of 100–150 μ g, through well tolerated according to other investigations, has not been generally exceeded, since we did not find the clinical opportunity of larger amounts. The total number of intrav. injections usually ranged between 10 and 20. Generally, the treatment has been interrupted after ascertained improvement.

RESULTS

a) *Bronchial asthma.*

1) W. P., a 30 years old woman. She has been suffering from asthma for 5 years (the disease had started during pregnancy); the crises occur especially at night. Hypersensitivity towards crustacea and strawberries. Sanamicina treatment (initial dose 40 μ g, then 80 μ g, 16 intrav. injections in total) induces rapid improvement and complete disappearance of the asthmatic symptomatology. Such improvement is still present 3 months later.

2) M. M., a 40 years old woman. She has been suffering for over 20 years from obstinate and prolonged crises of bronchial asthma, unresponsive to the most varying treatments. Sanamicina therapy (80 μ g initially, and 120 subsequently, a total of 10 intrav. injections) induces a significant improvement and disappearance of asthmatic symptomatology which are still present 3 months later.

3) S. L., a 23 years old man. Asthma for about 10 years, attacks occurring especially at night, and seasonal relapses (Autumn). Sanamicina treatment (18 intrav. injections, μg 80 to μg 100) induces a gradual decrease of the asthmatic crises, the respiration becomes easier and catarrh diminishes. The patient complains of dyspeptic troubles during the treatment. The improvement is still present 2 months later.

4) R. F., 59 years old. Chronic bronchitis for more than 20 years, with emphysematous and asthmatic complications. Co-existence of hypotension. A 80 μg dose of Sanamicina (6 intrav. injections) does not effect any improvement and the patient stops treatment.

5) S. M., a 35 years old man. Asthma for about 10 years caused especially by living in a dusty ambient. Sanamicina treatment (15 intrav. injections, 40 μg to 100 μg) induces a rapid and continuous improvement with disappearance of the most severe troubles. 1 month later such satisfactory condition is still observed.

6) M. M., a 47 years old man. He suffered from bronchitis complicated by bronchial asthma. Sanamicina (40 μ to 80 μg) determines initially a definite worsening. After a few days' interval, the patient was submitted again to intrav. injections with a daily dose of 80 μg . The treatment is now better tolerated: a rapid improvement is observed, and most troubles have been eliminated.

7) B. I., a 45 years old man. Asthma for about 15 years, with obstinate and prolonged attacks. Sanamicina therapy, 40 μg to 80 μg , induces in a few days a significant improvement of the symptomatology.

b) *Urticaria.*

1) D. C., a 32 years old woman. She complains of long periods of urticaria, occurring after meals, especially during Autumn and Spring. Sanamicina therapy (80 μg to 100 μg , 7 intrav. injections) induces a rapid disappearance of the urticaria crises, replaced by an irregular occurrence of erythematous spots without pruritus. During Sanamicina therapy the patient complains of gastric pains and cephalaea.

2) P. I., a 38 years old woman. She has been suffering for a few years from obstinate urticaria, especially when feeding consists chiefly of bread and paste. Sanamicina treatment (40 μg to 100-150 μg , 20 intrav. injections) induces a significant improvement of pruritus. Big but less disturbing pomphi follow the fine pruriginous eruptions. Food is better tolerated, and the improvement is still observed three months later.

3) S. A., a 46 years old man. Congelation urticaria for about 30 years. Erythema, pruritus and urticaria following body exposure to low temperatures. Only 5 intrav. injections of Sanamicina, 40 μg to 150 μg , are practised. Ice applications are then performed; no cutaneous reactions were noticed even after prolonged exposure. However, 1 month only after interruption of treatment the primary symptomatology reappeared.

4) M. A., a 46 years old woman. Urticaria regularly occurring after meals for more than 20 years. Sanamicina treatment (7 injections, 40 μ g to 120 μ g) induces a rapid improvement which is still present 3 months later.

5) C. M., a 35 years old woman. Urticaria after meals and at night. After 6 intrav. injections of Sanamicina (40 μ g to 120 μ g) a rapid improvement and disappearance of troubles are observed, which still prevails 3 months later.

6) S. A., a 52 years old woman. Urticaria for more than 4 years, especially consistent with ingestion of usual food. 18 intrav. injections of Sanamicina, 40 μ g to 200 μ g, are given. The symptomatology gradually decreases until disappearance of the most significant troubles. Co-existing painful rheumatismal crises are likely to be eliminated. Such good conditions are still present 3 months later.

7) A. D., a 46 years old man. He has been suffering for 6 years from genital pruritus occurred after sulfonamide ingestion and unresponsive to any treatment. The symptomatology is not modified by Sanamicina therapy (7 intrav. injections). Treatment is stopped because of a worsening of cholecyst troubles.

c) *Colitis*.

1) A. L., a 32 years old man. Chronic colitis, especially following intolerance towards some aliments. 13 intrav. injections of Sanamicina, 40 μ g to 200 μ g, determined a diminution of diarrheal discharges, but 1 month later the troubles reappeared.

Some biological changes induced in man by Actinomicina therapy have been studied by us and co-workers. The following results have been obtained:

General conditions.—The therapeutical treatment with C Actinomycin, in doses ranging from 40 to 200 μ g and for a period from 5 to 20 days, did not produce any particular condition of suffering. In general the drug has been tolerated well. In some cases abdominal pains were experienced in epigastric, colic, or in the hepato-cholecystic region.

Hematological picture.—C Actinomycin therapy did not induce great variations in the number of red and white blood cells. A particular study was carried out on the count of eosinophil cells. When dealing with subjects suffering from allergic diseases, the number of eosinophilic cells was initially higher than the normal mean values. Sanamicina therapy has generally resulted in a considerable decrease of eosinophilia. As regards the differential blood count, a relative increase of

lymphocytes has been observed during, and at the end of, the antibiotic treatment.

Laboratory tests.—Glycemia and azotemia were not modified by Actinomycin therapy. Takata-Ara and De Maria tests were positive during treatment in a patient who had previously suffered from hepatic disease. No significant modifications were seen in urine analysis.

Cutaneous reactions.—Tuberculin and histamine cutaneous reactions were not modified by C Actinomycin therapy.

Plasmaproteins.—In a group of allergic patients submitted to C Actinomycin therapy, a comparative study has been carried out on plasmaproteins before and after treatment. In most cases, an increase of total proteins resulted from Sanamicina therapy. Such increase was related to an increase of albumins, while globulins diminished. Of the globulin fractions, gamma-globulins showed the most significant decrease.

CONCLUSIONS

The chief lymphotropic action of Actinomycin and the correlation between the lymphatic system and immunity suggested an investigation on the possibility of a possible C Actinomycin action both on anaphylaxis and allergic syndromes. From the above mentioned, the conclusion may be drawn that C Actinomycin is unable to protect guinea-pigs against lethal crisis following ovalbumin re-injection. It is, however, to be observed that in animals treated with C Actinomycin the duration of the crisis was longer, and death delayed. Cortisone, too, is unable to protect against anaphylactic shock, while azo-hyprites appear to be more active. Such a difference in activity may well be ascribed to the stronger action of azo-hyprites on tissues, and consequently may produce trouble in the normal biological activities. In our experience, Sanamicina failure in protecting guinea-pigs against anaphylactic shock may be ascribed to a probable incomplete lesive interference with the antibiotic on the centers that are active in antibody formation and storage.

On the contrary, C Actinomycin was found to be therapeutically active in human allergy. Low daily doses of Sanamicina (generally 40 μ g to 150 μ g) intrav. administered for a maximum period of about 3 weeks, gave a good percentage of probative results. In fact, out of 7 cases of bronchial asthma very good results have been obtained in two, good in three, moderate in one, of no effect in one. Out of 7 cases of urticaria, results have been very good in three, good in two, moderate in one, of no effect in one. In one case of colitis moderate results were obtained. Even some inveterate and obstinate cases of bronchial asthma have been favourably influenced. Striking results have sometimes been noticed in chronic urticaria. The doses employed did not induce particular disturbances during treatment, except for an irregular occurrence of gastro-intestinal pains. However, the existence of an inflammatory cholecystopathy, frequently observed in guinea-pigs post-mortem examinations, lead one to advise a suitable diet and therapeutical measures during C Actinomycin treatment in patients suffering from liver diseases.

From the results obtained by these preliminary researches, it may be deduced that C Actinomycin can be further experimented with in the therapy of allergic diseases. There seems to be particular indications in favour of treating cases of hypersensitivity syndromes in conjunction with phlogistic foci, as e.g. in asthmatic bronchitis. Here Actinomycin may effectively develop two concomitant actions, i.e. an antibiotic action against bacteria and an antiallergic one on lymphoid structures.

The therapeutical results referred to also suggest the use of Actinomycin in rheumatism, due to its close relation with dysergic phenomena. While dealing with this subject, the remarkable improvement of rheumatic sympatopatology observed by us during Sanamicina therapy is noteworthy.

The study of this new antibiotic is worthy of further development. Histological investigations that are likely to elucidate many aspects of the interesting biological actions of C Actinomycin are in progress.

The study of the action of Actinomycin carried out both on guinea-pigs and allergic patients, led to the following deductions: C Actinomycin therapy is usually tolerated well. Relatively small doses induce in animals a transitory loss of weight. In human subjects, gastro-intestinal pains may be observed. A noteworthy finding is represented by a considerable dilatation and inflammation of the gall bladder often noted at post-mortem examinations of guinea-pigs. The administration of hepatoprotective drugs during C Actinomycin therapy in man is advisable to prevent recrudescence of hepatic troubles.

The absence of variations in the number of red and white blood cells, in general, has already been observed by earlier authors, and this gives us cause for preoccupation about the hematological conditions. It is to be noted that the numerical decrease of eosinophilic leukocytes, as observed in allergic patients during Sanamicina therapy, is in accordance with the favourable results due to the therapy itself. The increase of lymphocytes is very likely in relation with the elective lymphotropic action of C Actinomycin: the antibiotic exerts a stress action on the lymphatic tissues, mobilizing from them an abnormal number of lymphocytes which pass into the blood. This exceptional number of lymphocytes, very compensates the reduced transition of granulocytes from marrow to blood, due to the cytostatic action of Actinomycin.

The normal values of the various hematic components and the normal urinary finding, further prove the harmless action of this antibiotic.

The results obtained by electrophoretical examination of plasma-proteins are particularly noteworthy. The investigations carried out both on guinea-pigs and allergic patients show that C Actinomycin induces a decrease in globulins and particularly in gamma-globulins which, as generally known, are at present regarded as the carriers of antibodies. The significance of such a decrease may be regarded as follows: the damaging action of Actinomycin on lymphoid tissues, which is recognized as the principal seat of antibody-forma-

tion, prevents this important activity of the normal function of lymphoid tissues. Hence, the lymphoid histological damage is accompanied by a decreased level of gamma-globulins and consequently of antibodies. The diminution of antibodies may be a humoral justification of the improvement usually observed in allergic patients during Sanamicina treatment. Owing to reduced amount of antibodies, antigens are deprived of a considerable rate of reactive material, thus improving the clinical condition.

SUMMARY

The anti-anaphylactic and anti-allergic action of C Actinomycin (Sanamicina) has been studied. In guinea-pigs, the antibiotic did not protect against death following anaphylactic shock from egg albumin, though it exerted a certain influence on the average time of the crisis. On the contrary, an evident anti-allergic action, resulting in favourable therapeutic effects, has been observed both in bronchial asthma and urticaria.

As regards the biological actions of C Actinomycin in guinea-pigs and human beings, they may be summarized as follows:

Administration of C Actinomycin in guinea-pigs and allergic patients has usually been tolerated well. A tendency to loss of hair and cholecyst complications has, however, been encountered in guinea-pigs. In human beings, some troubles of the digestive apparatus were noted. As far as the hematological picture is concerned, the number of white and red blood cells was found to be practically unchanged. In most cases, a relative lymphocytosis was observed, very likely due to the lymphotropic action of C Actinomycin, and to a simultaneous cytostatic activity of the marrow. A significant decrease of eosinophil cells was found in allergic patients.

The study of plasmaproteins gave evidence of a constant diminution of globulins, and particularly of gamma-globulins. The improvement induced in allergic diseases by Sanamicina

therapy is attributable to such diminution, this is very likely due to the harmful activity of C Actinomycin on the lymphoid tissues which are supposed to be the seat of antibody-genesis.

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