RESEARCH PAPERS

THE ANTI-INFLAMMATORY ACTIVITY OF GLYCYRRHETINIC ACID AND DERIVATIVES

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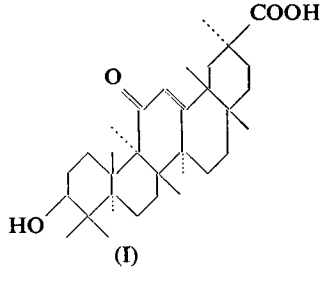
Received June 9, 1958

The anti-inflammatory activities of different fractions of glycyrrhetinic acid or glycyrrhetic acid and some of its derivatives have been assessed in laboratory animals. Some, but not all, preparations have been found to be active using four established methods for testing anti-inflammatory drugs. The findings provide a scientific basis for the clinical use of these compounds in inflammatory diseases, and may explain the discrepancies in the early clinical trials with this drug.

LIQUORICE has been used medicinally for generations, mainly as a demulcent and sweetening agent. It has only recently been discovered that some of its derivatives have an anti-inflammatory action. Revers[1] found that large doses of liquorice extract were effective in the treatment of stomach ulcers, and this was confirmed by Molhuysen and others[2]. The main water soluble constituent of liquorice is "glycyrrhizin", from which the aglycone "glycyrrhetinic acid" may be obtained by hydrolysis. The main component of crude glycyrrhetinic acid is the 18 β form, having the structure and configuration (I).



(I)

Glycyrrhetic Acid
Glycyrrhetinic Acid

The structure shows a resemblance to that of hydrocortisone, but the acid has no glucocorticoid action[3].

A number of isomers have been described[4]. We have found that not all fractions of glycyrrhetinic acid have an anti-inflammatory activity. This fact might account for the conflicting results of the early clinical trials with this compound in skin diseases. Adamson and Tillman[5] reported on the use of glycyrrhetinic acid in skin diseases. A number of small-scale trials followed with adverse results[6–8]. These may have been due to the

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material used, for the method of extraction is important. Evans[9], Annan[10], Chakravorti[11], Sommerville[12] and Colin-Jones and others[13,14], using materials which we had found to be biologically active, proved that they had valuable anti-inflammatory properties in a variety of dermatoses. Encouraging results have also been obtained in veterinary practice[15–18].

Similar conclusions have been made by Benigni and Franco[19] who found that the 18 β and so-called 18 γ isomers of glycyrrhetinic acid were active bacteriostatic agents while the 18 α form was inactive.

Little is known about the mode of action of glycyrrhetinic acid. But, Atherden[20] has shown that it inhibits the metabolism of progesterone and 11-deoxycorticosterone by rat-liver homogenates.

There have been few publications on the anti-inflammatory activity of glycyrrhetinic acid in induced inflammatory conditions using laboratory methods. Cornforth and Long[21] showed that certain fractions of liquorice suppressed, like hydrocortisone, the tuberculin reaction in B.C.G. sensitised guinea pigs. Somers[22] found glycyrrhetinic acid to be active in the cotton pellet test of Meier and others[23] and this was confirmed by D'Arcy and Kellett[24] and by Logemann, Lauria and Tosolini[25]. This paper describes the anti-inflammatory activity of glycyrrhetinic acid by four established methods for assessing anti-inflammatory drugs using laboratory animals. They prove that some, but not all, fractions of glycyrrhetinic acid are active anti-inflammatory agents, thus providing a scientific basis for the use of the active fractions in inflammatory conditions.

Materials

The samples of glycyrrhetinic acid and derivatives were those which had been prepared by Professor E. E. Turner, F.R.S., from material supplied through the courtesy of Dr. S. Gottfried of Biorex Laboratories, Ltd. Cortisone and hydrocortisone were used as saline suspensions in the form of the commercial preparations of the acetates Cortelan and Cortef.

Methods

The anti-inflammatory activity of glycyrrhetinic acid has been determined experimentally in animals by the following methods.

The Cotton Pellet Method of Meier, Schuler and Desaulles[23]

Small cotton wool pellets, when implanted under the skin of the rat become infiltrated with granulation tissue. An anti-inflammatory drug reduces the deposition of this granulation tissue so that the increase in weight of the pellet is reduced. Four cotton wool dental pellets weighing about 5 mg. are weighed and implanted under the skin of the anaesthetised rat, one in each groin and axilla. These pellets are left *in situ* for 6 days, the rats being injected daily subcutaneously or intramuscularly with the preparation under test. The rats are then killed, the pellets removed, extraneous tissue trimmed off and the pellets dried overnight in an oven at 60°. The pellets are again weighed and the amount of granulation tissue is taken as the difference between the initial and final weights. Groups of male wistar rats weighing about 180 g. are used for each

preparation and compared with uninjected controls. The mean gain in weight for the pellets in each group is calculated together with its standard error. A statistical assessment of the significance is made by the Students "*t*" test.

Inhibition of the Tuberculin Reaction in B.C.G. Sensitised Guinea Pigs

The method was first described by Long and Miles[26], and Cornforth and Long[21], who found that certain fractions of liquorice extract were as active as cortisone. We have modified the technique slightly to allow for variations in the response in different areas of the skin. White male guinea pigs are sensitised to tuberculin by injecting, intradermally, 3 weeks previously, 0·2 ml. of a 1 in 20 dilution of B.C.G. vaccine. For the test they are randomly distributed into groups of four, and injected subcutaneously, three times a day, for 5 days with the preparation under test. On the sixth day 0·2 ml. of a dilution of Tuberculin B.P. in saline is injected intradermally, into the previously depilated skin on the back at three dose levels, (*a*) 100 units/ml., (*b*) 400 units/ml. and (*c*) 1600 units/ml. The doses are injected into the different areas of the back in a randomised order according to a 3 × 3 Latin Square design[27]; each guinea pig giving three responses at each of the three dose levels. On the following day the diameters of the wheals are measured in the groups and the means plotted against the logarithm of the doses.

The Rat Foot Test

The method was described by Selye[28]. For the test, groups of rats are injected subcutaneously daily for 6 days with the test drug, and on the last day 0·2 ml. of a 3 per cent solution of formaldehyde is injected into the plantar aponeurosis of the right rear foot. The degree of swelling is determined on the following day by comparing the volume of the injected foot with the volume of the uninjected foot, using the microburette described by Buttle and others[27]. The response is the difference between the volume of the injected and the uninjected foot and when expressed as a percentage of the uninjected foot may be compared with the percentage increase of the control.

The Granuloma Pouch Method

This method was also described by Selye[30]. For the test, 25 ml. of air is slowly injected through a fine hypodermic needle under the skin of the back of the rat. Into the air sac which is formed, there is injected 0·5 ml. of a 1 per cent solution of croton oil in arachis oil. A suspension of the drug being tested is injected subcutaneously daily for 5 days, in another site. The rat is killed on the sixth day and the pouch dissected. An anti-inflammatory drug reduces the thickening of the wall of the pouch and the exudation of fluid into the sac.

RESULTS

Cotton Pellet Method

Glycyrrhetinic acid depressed the formation of granulation tissue induced by subcutaneously implanted cotton pellets in rats. The response

is related to the dose[31] (Table I). Providing the dose was sufficient, glycyrrhetinic acid was as active as hydrocortisone. Figure 1 shows the results of a typical comparison of glycyrrhetinic acid and hydrocortisone in this test. Weight for weight glycyrrhetinic acid is between $\frac{1}{5}$th and $\frac{1}{10}$th

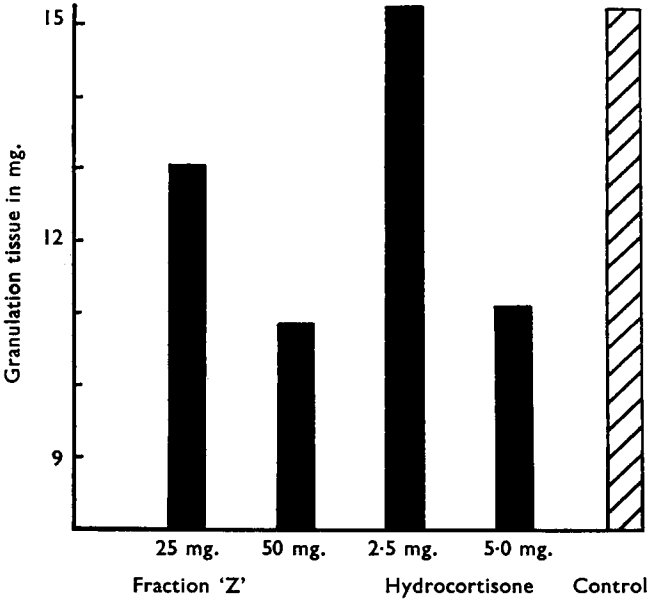

FIG. 1. The effect of glycyrrhetinic acid Fraction 'Z' and hydrocortisone on the formation of granulation tissue in cotton wool pellets.

the activity of hydrocortisone. However, the low solubility of glycyrrhetinic acid is a limiting factor, much of the injected material remaining at the site of the injection. It is for this reason that no direct quantitative comparison with hydrocortisone can be validly made. A number of derivatives of glycyrrhetinic acid were tested and the glycyrrhetinic acid

TABLE I

DEPRESSION OF FORMATION OF GRANULATION TISSUE BY GLYCYRRHETINIC ACID (FRACTION D) IN THE COTTON PELLET TEST IN THE RAT

Treatment	No. of rats	Granulation tissue mg.	Standard error mg.	Per cent of controls
Controls	10	14·1	±0·471	—
Glycyrrhetinic acid Fraction "D" 6·25 mg. ..	10	12·4	±0·636	88
Glycyrrhetinic acid Fraction "D" 12·5 mg. ..	10	11·3	±0·393	80
Glycyrrhetinic acid Fraction "D" 25 mg.	10	10·1	±0·608	72
Glycyrrhetinic acid Fraction "D" 50 mg.	10	9·4	±0·649	67

hydrogen succinate, the glycyrrhetinic acid propionate, the piperazine salt of glycyrrhetinic acid and the acyl derivative were found to be active (Tables II and III). The soluble sodium salt of the glycyrrhetinic acid hydrogen succinate was particularly active as seen in Table IV.

Inhibition of the Tuberculin Reaction in B.C.G. Sensitised Guinea Pigs

The results of a typical experiment are shown in Table V. They show that doses of 5 mg./kg. of glycyrrhetinic acid injected subcutaneously suppressed the tuberculin reaction in the guinea pig in the same way as 4 mg./kg. of cortisone when compared with untreated controls. Similar results were obtained with certain fractions of liquorice by Cornforth and

TABLE II

DEPRESSION OF THE FORMATION OF GRANULATION TISSUE BY A NUMBER OF DERIVATIVES OF GLYCYRRHETINIC ACID IN THE COTTON PELLET TEST IN THE RAT

Treatment	No. of pellets	Granulation tissue mg.	Standard error mg.	Per cent of controls	Significance to controls
Controls—saline	48	9·5	±0·65	—	—
Hydrocortisone 2·5 mg.	24	5·6	±0·27	59	P <0·001
Glycyrrhetinic acid hydrogen succinate 12·5 mg.	24	6·0	±0·028	63	P < 0·001
Glycyrrhetinic acid propionate 12·5 mg. ..	24	6·7	±0·38	70	P = 0·001
Glycyrrhetinic acid Fraction "Z" 12·5 mg. ..	24	6·8	±0·4	72	P = 0·001
Piperazine salt of glycyrrhetinic acid 6·75 mg.	24	6·8	±0·39	72	P = 0·0015

TABLE III

DEPRESSION OF THE FORMATION OF GRANULATION TISSUE BY A NUMBER OF PREPARATIONS AND DERIVATIVES OF GLYCYRRHETINIC ACID IN THE COTTON PELLET TEST IN THE RAT

Treatment	No. of pellets	Granulation tissue mg.	Standard error mg.	Per cent of controls	Significance to controls
Controls—saline	16	11·0	±0·85	—	—
Glycyrrhetinic acid methyl ester 12·5 mg. ..	16	9·3	±0·83	85	P > 0·05
Glycyrrhetinic acid Fraction "D" 12·5 mg. ..	16	8·3	±0·54	75	P = 0·02
Glycyrrhetinic acid Fraction "S" 12·5 mg. ..	16	8·6	±0·62	78	P = 0·05
Acyl derivative of glycyrrhetinic acid 12·5 mg.	16	7·6	±0·45	69	P = 0·005

TABLE IV

DEPRESSION OF THE FORMATION OF GRANULATION TISSUE BY THE SODIUM SALT OF GLYCYRRHETINIC ACID HYDROGEN SUCCINATE IN THE COTTON PELLET TEST IN THE RAT

Treatment	No. of pellets	Granulation tissue mg.	Standard error mg.	Per cent of controls	Significance to controls
Controls	16	13·3	±0·34	—	—
Sodium salt of glycyrrhetinic acid hydrogen succinate 12·5 mg.	16	7·8	±0·19	58·6	P < 0·001

Long[21]. They have stated, however, that this activity was not due to "glycyrrhetinic acid" which they found to be inactive in the test[32]. This discrepancy could be due to the material they used, for not all samples of so-called "glycyrrhetinic acid" are active in the test. We have ourselves tested a sample of "commercially pure" glycyrrhetinic acid and found it to be inactive in the test (Table VI).

Rat Foot Test

Glycyrrhetinic acid was active in this test when given daily for 6 days before the injection of formaldehyde into the foot. Table VII shows the

results obtained in comparison with hydrocortisone. A greater depression of the swelling was obtained with increasing doses of glycyrrhetinic acid, but this was not so with hydrocortisone where increasing the dose decreased the protection. Similar findings were made with hydrocortisone

TABLE V

INHIBITION OF TUBERCULIN REACTION IN B.C.G. INJECTED GUINEA PIGS

Treatment	No. of wheals per dose	Mean wheal diameter in mm. Units of tuberculin 20	80	320	Significance to controls Units of Tuberculin 20	80	320
Controls	12	11	16	18·5	—	—	—
Cortisone 4 mg./kg. ..	12	11	13	15	The inflammatory response was too small to demonstrate the anti-inflammatory activity of the drug	P = 0·02	P = 0·01
Glycyrrhetinic acid Fraction "D" 5 mg./kg. ..	12	10·5	12·5	15		P = 0·01	P = 0·01

TABLE VI

INHIBITION OF TUBERCULIN REACTION IN B.C.G. INJECTED GUINEA PIGS

Treatment	No. of wheals per dose	Mean wheal diameter in mm. Units of Tuberculin 20	80	320
Controls	12	7·0	9·3	12·2
Cortisone 5 mg./kg.	12	6·0	6·6	9·0
Glycyrrhetinic acid Fraction "Y" 25 mg./kg.	12	8·3	9·8	14·1

TABLE VII

REDUCTION IN SWELLING OF RAT FOOT INJECTED WITH FORMALDEHYDE BY GLYCYRRHETINIC ACID FRACTION "Z" AND HYDROCORTISONE

Treatment and dose per 200 g. rat	No. of rats	Mean volume of foot ml. Left	Right	Mean difference ml. and standard error	Mean increase as per cent of controls
Control	4	1·50	1·97	0·47 ± 0·07	—
Glycyrrhetinic acid Fraction "Z" 25 mg.	4	1·52	1·90	0·38 ± 0·11	81
Glycyrrhetinic acid Fraction "Z" 50 mg.	4	1·37	1·67	0·30 ± 0·07	64
Glycyrrhetinic acid Fraction "Z" 100 mg.	4	1·45	1·70	0·25 ± 0·08	53
Hydrocortisone 2·5 mg.	4	1·45	1·60	0·15 ± 0·03	32
Hydrocortisone 5·0 mg.	4	1·52	1·80	0·28 ± 0·09	59
Hydrocortisone 10·0 mg.	4	1·55	1·90	0·35 ± 0·06	75

by Cooper and others[33], who reported that after repeated doses of hydrocortisone the oedema produced by silver nitrate injected into the paw of the mouse was increased.

Granuloma Pouch Method

The effects here were dramatic, but difficult to assess quantitatively. In the control rats the croton oil caused gross inflammation in the sac. The wall was thickened and the blood vessels engorged. The sac contained a light brown fluid often blood stained and the underlying tissues showed

necrotic changes with pus formation. With hydrocortisone and glycyrrhetinic acid these changes were prevented, the wall of the sac remained thin and only slightly inflamed, there was no fluid in the sac and no necrosis of the underlying tissue (Table VIII).

TABLE VIII

ANTI-INFLAMMATORY ACTION OF HYDROCORTISONE AND GLYCYRRHETINIC ACID IN THE GRANULOMA POUCH TEST

Treatment	No. of rats	Mean volume fluid ml.	Result
Controls	8	1·3	Sac contained inflammatory exudate. Wall of sac thickened and grossly inflamed
Glycyrrhetinic acid Fraction "Z" 12·5 mg.	4	2·0	do.
Glycyrrhetinic acid Fraction "Δ" 12·5 mg.	4	0	Sac thin and only very slightly inflamed. No fluid present
Glycyrrhetinic acid Fraction "Δ" 5·0 mg.	4	0	
Glycyrrhetinic acid Fraction "Δ" 1·25 mg.	4	0	
Hydrocortisone 5 mg.	4	0	do.
Hydrocortisone 1·35 mg.	4	0	
Hydrocortisone 0·35 mg.	4	0	

CONCLUSIONS

These results, obtained in four different kinds of tests, show that glycyrrhetinic acid is an active anti-inflammatory agent. They have been obtained in carefully controlled experiments with laboratory animals where psychological factors can presumably be ruled out. In three of these tests the responses have been assessed by actual measurement and not by subjective comparisons which may be influenced by the observer. The statistical validity of the findings has been proved and they provide a scientific foundation for the use of the biologically active fractions of glycyrrhetinic acid in inflammatory conditions and explain the clinical effectiveness of the drug.

Acknowledgements. The authors are indebted to Professor E. E. Turner, F.R.S., of Bedford College, University of London, and Dr. S. Gottfried of Biorex Laboratories, Ltd., London, E.C.1, for their help and generous supply of materials.

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