J. Pharm. Pharmacol. 1984, 36: 820–823
Received April 3, 1984

Anti-inflammatory and anti-ulcer properties of hypolaetin-8-glucoside, a novel plant flavonoid

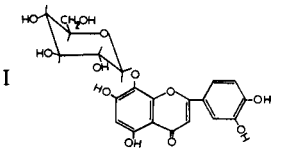
A. VILLAR, M. A. GASCO AND M. J. ALCARAZ*

Department of Pharmacognosy and Pharmacodynamics, Faculty of Pharmacy, Avda Blasco Ibañez 13, Valencia-10, Spain

The anti-inflammatory, analgesic and anti-ulcer activities of a novel flavonoid, hypolaetin-8-glucoside, obtained from *Sideritis mugronensis*, have been tested in the rat. The flavonoid was more potent than phenylbutazone in suppressing the acute phase of adjuvant-carrageenan-induced inflammation, but had less effect in the prolonged inflammatory phase. However, unlike phenylbutazone, it did not cause gastric erosions. Both compounds were equiactive in inhibiting the development of carrageenan-induced abscesses, whereas phenylbutazone had greater analgesic activity in tests on pressure pain threshold. The flavonoid and cimetidine both prevented the formation of cold-restraint induced gastric lesions, but cimetidine was more potent. These results show that hypolaetin-8-glucoside combines both anti-inflammatory and anti-ulcer properties and suggest that it may offer useful alternatives to anti-inflammatory drugs of the aspirin type.

Extracts from various species of the genus *Sideritis* (*Lamiaceae*) are used in Spanish folk remedies as anti-inflammatory and anti-rheumatic agents and have also been credited with exerting beneficial effects on the alimentary tract. Previously it was shown that a diterpenoid obtained from *Sideritis mugronensis*, borjatriol, possessed anti-inflammatory properties (Soler, 1980; Villar et al 1983, 1984b) and that various flavonoid-containing extracts from this plant were also active (Villar et al 1982a). In the present paper we demonstrate that a purified flavonoid obtained from *S. mugronensis* (Villar et al 1982b), and identified as hypolaetin-8-glucoside (I; Villar et al 1984a), exerts both anti-inflammatory and antigastric ulcer actions in various animal models.

I



MATERIALS AND METHODS

Extraction and purification of hypolaetin-8-glucoside

Aerial parts of *S. mugronensis* were collected from the Bonete area (Albacete province, Spain) in July 1981, and a voucher specimen has been deposited in the herbarium, Department of Botany, Faculty of Pharmacy, Valencia.

* Correspondence to present address: Department of Pharmacology, King's College, Strand, London WC2R 2LS, UK.

After drying and grinding, the plant material (10 kg) was defatted with light petroleum (bp 50–70 °C) and then extracted with methanol. The concentrated methanolic extract was suspended in distilled water and extracted successively with benzene and ethyl acetate. The flavonoid hypolaetin-8-glucoside (5, 7, 8, 3′, 4′ pentahydroxyflavone-8-B-D glucoside) (2 g) was purified from the ethyl acetate extract by repeated precipitations in methanol.

Assays for anti-inflammatory activity

Adjuvant-carrageenan-induced inflammation (ACII) was induced in male Wistar rats (150–170 g) using the technique of Mizushima et al (1972). For this, 0·1 ml Freund's complete adjuvant (Difco) was given intradermally into the basal part of the tail, followed 6 days later by 0·1 ml of 2% suspension in 0·9% NaCl (saline) of carrageenan (Marine Colloids Inc.) injected into the subplantar region of the left hindpaw. The volume of the injected paw was recorded plethysmographically before administration of carrageenan and 3, 24, 48 and 72 h later. Test drugs or vehicle were injected 1 h before and 24 h after carrageenan. At the end of the experiment the animals were killed and the stomachs removed, opened along the lesser curvature, washed and examined under a ×3 magnifier to assess the formation of ulcers using a 0 to 3 scale as described by Aparicio (1977). The ulcer score is expressed as a percentage based on a maximum value per group (6 animals) of 18.

Carrageenan-induced abscesses were induced in female Wistar rats (180–200 g) using a technique described by Benitz & Hall (1963). Under light ether

anaesthesia, 0·5 ml of a 2% suspension of carrageenan was injected s.c. in the dorsal area; the rats were killed 48 h later and the abscesses removed and weighed. Test drugs were injected immediately after the carrageenan administration and 24 h later.

Assay for analgesic activity

Analgesia was measured in rats in terms of the pressure threshold for pain in oedematous hindpaws injected with 0·1 ml of a 2% suspension of carrageenan (Rozskowski et al 1971). Male Wistar rats (150–180 g) were used, and the tests were made 1 h after induction of oedema. Test drugs were administered 1 h before injection of carrageenan.

Cold restraint-induced ulcers

Male Wistar rats (190–200 g) were allowed water but deprived of food for 24 h before i.p. administration of the test compounds. The animals were then placed in individual cylinders of 4·5 cm diameter, 25 cm length, and were kept at 4 °C. After 4 h the rats were killed and the stomachs removed and prepared as described above. The diameters of the lesions were measured and the areas summed to give a total lesion score in mm^2 for each animal.

Drugs, statistics

Phenylbutazone (Geigy), cimetidine (Smith Kline & French) and hypolaetin-8-glucoside were injected i.p. in a volume of 0·5 ml using as vehicle 6% Tween 80 in distilled water containing 1% (w/v) methylcellulose. Control animals had injections of vehicle alone. Groups of 6 animals were used for each test, and the data are given as mean ± s.e.m., with differences between groups evaluated statistically using Student's unpaired *t*-test.

RESULTS

Adjuvant-carrageenan-induced inflammation

The inflammatory process in this experimental model involves two phases, with a first step (acute phase) which closely resembles that of carrageenan-induced paw oedema and is maximal 3 h after injection of the carrageenan, followed by a prolonged phase in which oedema recurs after 48–96 h (Mizushima et al 1972). Table 1 compares the anti-inflammatory activities of hypolaetin-8-glucoside and phenylbutazone in this model. Both compounds suppressed oedema in the acute phase in a dose-dependent manner, but the flavonoid was more potent. Calculated regression lines from this data yield ED50 values of 56·9 ± 4·4 mg kg^{-1} for the flavonoid and 85·2 ± 2·3 for phenylbutazone ($P < 0.001$).

Table 1 also shows that the anti-inflammatory actions of hypolaetin-8-glucoside, although remaining dose-related, declined with time such that the only statistically significant reduction of oedema was achieved by the highest dose at 24 h. In contrast, phenylbutazone exerted dose-related suppression of oedema in the prolonged phase of this model, and this was greatest at 48 h although still evident for the highest dose at 72 h.

As explained in Materials and Methods, the stomachs were examined at 72 h for the presence of ulcers. Table 1 shows that treatment with flavonoid did not cause gastric erosions whereas phenylbutazone was damaging, especially at 90 mg kg^{-1}.

Carageenan-induced abscesses

Fig. 1 shows that the flavonoid and phenylbutazone inhibited the development of carrageenan-induced abscesses in the rats in a dose-dependent manner when administered twice at the same doses used in

Table 1. Inhibition by hypolaetin-8-glucoside and phenylbutazone of adjuvant-carrageenan-induced inflammation.

Time after carrageenan (h)	Control Oedema, %†	Inhibition of swelling, %					
		Hypolaetin-8-glucoside (mg kg^{-1})			Phenylbutazone (mg kg^{-1})		
		30	60	90	30	60	90
3	138·5	37·1 ± 3·2*	43·8 ± 3·8*	74·2 ± 1·3**	17·9 ± 3·6	43·3 ± 2·2**	49·3 ± 0·9**
24	57·5	21·7 ± 3·2	24·3 ± 2·6	48·7 ± 4·2*	0	21·7 ± 3·6	32·4 ± 2·6
48	45·2	9·0 ± 3·8	0·4 ± 5·2	34·7 ± 5·4	31·4 ± 5·4	45·0 ± 4·0*	69·0 ± 2·8**
72	49·7	0	0	12·4 ± 5·2	3·0 ± 2·0	6·0 ± 2·5	31·2 ± 3·2*
Per cent ulcerogenicity at 72 h	5·5	5·5	0	0	11·1	16·6	33·3

† Control values show percent increases in paw volume compared to those obtained before carrageenan injection in animals pretreated with vehicle alone. (Results are mean ± s.e.m., n = 6, *$P < 0.05$, **$P < 0.01$.)

the previous experiment. There was no significant difference in the potencies of the two agents which gave ED50 values of 59·2 ± 8·7 mg kg^{-1} for hypolaetin-8-glucoside and 51·6 ± 10·3 mg kg^{-1} for phenylbutazone, and the dose-response lines were parallel.

Analgesic activity

Both phenylbutazone and the flavonoid behaved as analgesics in that the threshold for pain sensation in response to pressure applied to an oedematous paw was increased as a function of the doses administered (Fig. 1). Both agents were ineffective at the lowest dose tested (30 mg kg^{-1} i.p., 2 h before the pressure test) whereas at the two higher doses phenylbutazone was significantly more effective, and the dose-response regression lines for the two drugs were not parallel (Fig. 1).

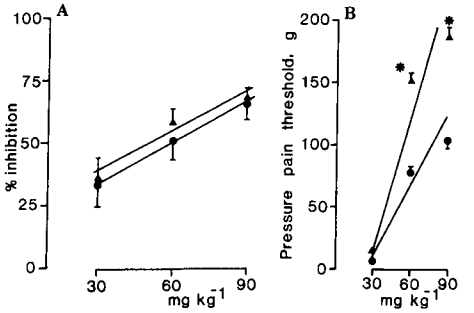


FIG. 1. Comparison in rats of the ability of hypolaetin-8-glucoside (●) and phenylbutazone (▲) to prevent the development of carrageenan-induced abscess (A) and to increase the threshold for pain (B). Basal values for the 48 h growth of an abscess in response to carrageenan and the threshold sensitivity to pain in a hindpaw injected 1 h previously with carrageenan were 3·9 ± 0·4 mg and 88·3 ± 6·4 g, respectively. Drugs were administered i.p. according to the schedules outlined in materials and methods. Results show mean ± s.e.m. for 6 animals and * indicates that this result for phenylbutazone is significantly different from that for flavonoid at the same dose, $P < 0·01$.

Cold-restraint induced ulcers

Hypolaetin-8-glucoside was compared with cimetidine for its ability to prevent the formation of gastric lesions in the stomachs of starved rats subjected to 4 h cold-restraint stress. Both compounds exerted strong protective effects which were significant at two doses tested (Table 2), but cimetidine was considerably more potent with a calculated ED50 value of 23·9 ± 11·8 mg kg^{-1}, compared with 57·3 ± 11·7 mg kg^{-1} for the flavonoid.

DISCUSSION

These results establish that hypolaetin-8-glucoside, a novel flavonoid which we have extracted and purified from *Sideritis mugronensis*, has potent anti-inflammatory, analgesic and anti-ulcer actions, at least in the rat. This is the first report of the biological actions of this flavonoid, although the properties we have identified have been noted in a rather scattered literature for other related flavonoids. For example, Gupta (1971), Il'Yuchenok (1975), Anand et al (1978) and Agarwal (1982) have defined anti-inflammatory actions of certain flavonoids, and Ciaceri & Attaguile (1972) and Ilarianov et al (1979) have observed protection against ulcers.

Our results may help also to establish a scientific basis for the use in Spanish folk medicine of crude plant extracts of *S. mugronensis* as an anti-rheumatic, anti-inflammatory and digestive agent. Nevertheless, the flavonoid is not the only pharmacologically relevant constituent; we have previously found that a terpenoid present in this plant (borjatriol) also has anti-inflammatory actions (Villar et al 1983, 1984b). Nevertheless, it does not produce analgesia (Soler 1980).

An important feature of flavonoids in general (see Havsteen 1983) and extracts of *S. mugronensis* (Esplugues et al 1982) and hypolaetin-8-glucoside in particular, is their low toxicity. In tests on albino mice, we found that doses of hypolaetin-8-glucoside up to 600 mg kg^{-1} (about 10 times the ED50 values in the rat for suppression of acute phase oedema in the adjuvant-carrageenan-induced inflammation test) were non-toxic and we were unable to establish

Table 2. Protection by hypolaetin-8-glucoside and cimetidine against cold-stress induced gastric ulcers in the rat.

Percent reduction in area of lesions					
Hypolaetin-8-glucoside (mg kg^{-1})			Cimetidine (mg kg^{-1})		
30	60	90	40	70	100
34·7 ± 10·7	45·6 ± 5·6*	67·7 ± 4·4*	58·5 ± 7·0	81·3 ± 2·6**	95·9 ± 0·9**

Compounds were administered i.p. at the start of the 4 h stress period. Results are mean ± s.e.m., n = 6, and the symbols show statistical significance of differences with respect to control animals not treated with drugs (lesion score at 4 h of 1·1 ± 0·2 mm^2), *$P < 0·05$, **$P < 0·01$.

a value for the LD50 as insufficient material was available to achieve the very large doses that would have been needed.

The inhibition of the acute 3 h swelling in the ACII test reflects the effectiveness of hypolaetin-8-glucoside on the acute inflammatory response, and the ED50 is similar to the value we reported previously in the carrageenan foot paw oedema test (Villar et al 1982b). The flavonoid appears to exert a more pronounced protective effect on the acute phase than on the prolonged phase in this animal model (and, indeed, phenylbutazone was less effective than anticipated from the results of Mizushima et al 1972); this means that extrapolation of the benefit of hypolaetin-8-glucoside to conditions involving chronic inflammatory changes cannot be made confidently, and suggest that the flavonoid should be tested in other animal models of chronic inflammation.

We also obtained evidence that hypolaetin-8-glucoside not only prevents oedema but is of comparable efficacy to phenylbutazone in inhibiting the development of granulation tissue in the carrageenan-abscess model (Fig. 1). However, it was somewhat less potent as an analgesic (Fig. 1). These findings might prompt the view that, pharmacologically speaking, this flavonoid represents another example of a non-steroidal aspirin-like agent. That this is clearly not the case is illustrated by our finding that, in contrast to phenylbutazone, hypolaetin-8-glucoside at anti-inflammatory doses did not induce the formation of microscopically-visible ulcers (Table 1). Indeed, in separate experiments (Table 2), it was shown that the flavonoid exerts clear protective effects against the induction in the rat of gastric ulcers induced by cold restraint, although it was not as potent as cimetidine.

The combination of anti-inflammatory and anti-ulcer actions is unusual but favourable. A well-known limitation of the large number of otherwise well tolerated aspirin-like anti-inflammatory analgesic agents is their tendency to produce gastric irritation, bleeding and mucosal cellular damage. To some extent at least, this may be related (Whittle 1983) to their ability to act as inhibitors of prostaglandin biosynthesis (Vane 1971), a property which also explains their anti-inflammatory, antipyretic and analgesic actions (Ferreira & Vane 1979). In the case of the flavonoid, hypolaetin-8-glucoside, it is highly unlikely that this biochemical action underlies the observed pharmacological actions in-vivo. For example, in preliminary experiments using sheep seminal vesicle microsomes and rabbit kidney medulla microsomes (Alcaraz, M. J. & Hoult, J. R. S., unpublished observations), we have found no evidence that this flavonoid inhibits cyclo-oxygenase, although phenylbutazone and indomethacin actively did so. It is therefore necessary to find some other explanation for the anti-inflammatory actions of hypolaetin-8-glucoside, as well as to identify the reasons for its ability to protect the gastric mucosa from ulceration. Further experiments may also reveal whether the potentially beneficial combination of anti-ulcer and anti-inflammatory actions could be exploited in man more advantageously than is presently the case, using plant extracts in a non-medical context.

Acknowledgements

We thank the Spanish Ministry of Education for financial support, and Dr J. R. S. Hoult for advice on drafting of the manuscript.

REFERENCES

Agarwal, O. P. (1982) Agents and Actions 12: 298–302

Anand, K. K., Sharma, M. L., Ray Ghatak, B. J. (1978) Indian J. Exp. Biol. 16: 1216–1217

Aparicio, L. (1977) Arch Int. Pharmacodyn. 227: 130–141

Benitz, K. F., Hall, L. M. (1963) Ibid. 144: 185–191

Ciaceri, G., Attaguile, G. (1972) Minerva Med., 63: 1665–1667

Esplugues, J., Villar, A., Alcaraz, M. J. (1982) Plantes med. et Phytother. XVI: 137–146

Ferreira, S. H., Vane, J. R. (1979) in: Vane, J. R., Ferreira, S. H. (eds) Anti-inflammatory Drugs (Handbook of Experimental Pharmacology, 50: part II), pp 348–398, Springer-Verlag, Berlin

Gupta, M. B. (1971) Jpn. J. Pharmacol. 21: 377–380

Havsteen, B. (1983) Biochem. Pharmacol. 32: 1141–1148

Ilarianov, I., Rainova, L., Nakov, L. (1979) Farmatsiya 29: 39–44

Il'Yuchenok, A. I. (1975) Russian Pharmacol. Toxicol. 38: 213–220

Mizushima, Y., Tsukada, W., Akimoto, T. (1972) J. Pharm. Pharmacol. 24: 781–785

Rozskowski, A. P., Rooks, W. H., Tomolonis, A. J., Miller, L. M. (1971) J. Pharmacol. Exp Ther. 179: 114–123

Soler, M. A. (1980) Ph.D. Thesis, Valencia University

Vane, J. R. (1971) Nature, New Biol. 231: 232–235

Villar, A., Esplugues, J., Alcaraz, M. J. (1982a) Plantes med. et Phytother. XVI: 157–162

Villar, A., Esplugues, J., Alcaraz, M. J. (1982b) Arch. De Farmacol y Toxicol. VIII: 99–106

Villar, A., Gasco, M. A., Mañez, S., Cortes, D., Alcaraz, M. J. (1984a) Proceedings of 32nd International Congress on Medicinal Plant Research, Antwerp, July 1984

Villar, A., Salom, R., Alcaraz, M. J. (1983) Pharmazie 38: 566

Villar, A., Salom, R., Alcaraz, M. J. (1984b) Planta med. 50: 90–96

Whittle, B. J. R. (1983) Br. J. Pharmac. 80: 545–551