REVISTA ESPAÑOLA DE FISIOLOGIA, 34, 55-60. 1978

Lack of Correlation Between Antiinflammatory Activity and Inhibition of 3′,5′-Cyclic AMP Phosphodiesterase Activity in a Series of Antiinflammatory Agents

J. García-Rafanell, M. S. Sánchez, V. Rimbau and J. Forn

Centro de Investigación
de Laboratorios J. Uriach & Cía., S. A.
Decano Bahí, 59-67. Barcelona - 13
(España)

(Received on April 18, 1977)

J. GARCIA-RAFANELL, M. S. SANCHEZ, V. RIMBAU and J. FORN. *Lack of Correlation Between Antiinflammatory Activity and Inhibition of 3′,5′-Cyclyc AMP Phosphodiesterase Activity in a Series of Antiinflammatory Agents.* Rev. esp. Fisiol., 34, 55-60. 1978.

The effect of several non steroid antiinflammatory agents on beef heart phosphodiesterase has been studied. A comparison of this effect with the *in vivo* antiinflammatory activity determined in rat (Winter's method) and with the recommended clinical daily dose in rheumatoid arthritis, reveals a lack of correlation. The possibility that cyclic AMP plays an important rôle in the inflammatory response cannot be completely ruled out, but it can be safely concluded fron the present study that the measurement of the inhibition of beef heart phosphodiesterase by potential antiinflammatory agents cannot be used as a test to predict their antiinflammatory activity.

Several different hypothesis have been advanced to explain the mechanisms of action of non-steroidal antiinflammatory agents. Some of them include: inhibition of oxidative phosphorylation (17), interference with migration of leucocytes (2, 3), stabilization of lysosomal membranes (7) and prostaglandin biosynthesis inhibition (15).

Recently, several works have suggested that cyclic AMP is involved in the development of inflammation. Cyclic AMP inhibits the carragenin edema in rats and this effect is potentiated by teophylline (6). Otherwise, WEINRYB *et al.* (16) have studied the inhibition of rat brain and cat heart phosphodiesterase (PDE) by several antiinflammatories. The experiences carried out by STEFANOVICH (11) indicate that *in vitro* inhibition of PDE from several tissue could be used as a preliminary test system for antiinflammatory agents.

In this study, the effect on beef heart PDE activity of two new phenylalcanoic compounds, three new 2-hydroxybenzoic

acid derivatives, and other standard anti-inflammatory drugs has been investigated. The *in vitro* inhibition of PDE activity produced by these compounds has been compared with their *in vivo* antiinflammatory activity.

Materials and Methods

Chemicals. Titriated 3',5'-cyclic AMP (40 mCi/mmole) was purchased from New England Nuclear, and 3',5'-cyclic AMP from Boehringer Manheim. Snake venom *(Crotalus atrox)* was obtained from Sigma Chemical Co. The anion-exchange resin was Bio-Rad AG 1-X-8, Cl^-, 200-400 mesh. The resin was used in a suspension form with one volume of pure ethanol. The scintillation solvents were PPO (4 g), dimethyl POPOP (0.1 g), naftalene (80 g), dioxane (385 ml), xylene (385 ml) and pure ethanol (230 ml).

Indomethacin and aspirin were obtained from Impex Química and Iberdroga, respectively. Diclofenac sodium, flufenamic acid, ibuprofen, ketoprofen, mefenamic acid and naproxen were obtained by extraction from the corresponding pharmaceutical specialities. The new phenylalcanoic derivatives, UR-333 and UR-337, as well as the 2-hydroxy benzoic acid derivatives, UR-1501, UR-1522 and UR-1524, were synthetized in our laboratory. Figure 1 shows the chemical structure of the UR- series.

Enzyme preparation. Purified bovine heart PDE (specific activity: 0.1 U/mg, 10 mg/ml) (Boehringer Manheim) was diluted with 25 mM Tris-maleate buffer, pH = 7.4. Final concentration in the incubation medium was 10 μg/ml.

Phosphodiesterase assay. The enzyme activity was measured by the procedure of THOMPSON and APPLEMAN (12) with slight modifications. The standard incubation mixture contained 25 mM Tris-maleate pH 7.4. 5 mM $MgCl_2$ and 10 μg/ml

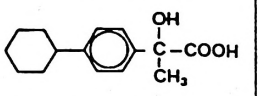
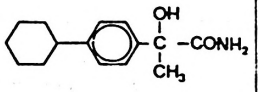
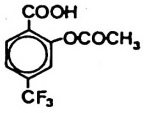
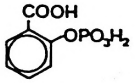
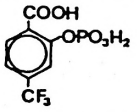
DENOMINATION	CHEMICAL STRUCTURE
UR-333	
UR-337	
UR-1501	
UR-1522	
UR-1524	

Fig. 1. *Chemical structure of the new anti-inflammatories tested.*

of heart phosphodiesterase. The antiinflammatory agents tested were dissolved in Tris buffer or ethanol, and in each case the same amount of solvent was added to the control samples. The enzymatic reaction was started by addition of 0.05 ml of 0.01 mM ^{3}H-cyclic AMP (approximately 50,000 cpm). Total final volume was 0.5 ml. After incubating for 10 min at 30° C, the reaction was stopped by placing the tubes in a boiling water bath for 2 min. Following cooling to 30° C, 0.1 ml of a 2 mg/ml aqueous solution of snake venom was added. After 30 min incubation at 30° C, the reaction was stopped by the addition of 0.5 ml of an alcoholic suspension (1:1, v/v) of Bio-Rad resin AG1-X8, 200-400 mesh. The samples were vigorously shaken for 10 min and then centrifuged at 3,000 rpm for 5 min. Aliquots of the supernatant were immediately taken for liquid scintillation counting.

Results

The inhibition of beef heart PDE activity induced by the antiinflammatory drugs studied is shown in figure 2. The concentration of each drug producing 50 % inhibition of PDE activity (I_{50}) has been calculated from the dose-reponse curves represented in figure 2. The I_{50} values are shown in table I. These values are compared in the same table with the antiinflammatory activity determined by the test of carragenin-induced edema in the rat (18) and with the clinical daily dose in rheumatoid arthritis (4, 9, 10).

Flufenamic acid and indomethacin are the most potent PDE inhibitors. Mefenamic acid, diclofenac sodium and UR-333 are the following in potency. A third group, with I_{50} in the milimolar range, incluyes UR-337, ketoprofen, UR-1524, UR-1522 and UR-1501. Aspirin is the least potent of all compounds studied with I_{50} of 26,000 μM.

Comparing I_{50} values with antiinflammatory activity (table I), no good correlations can be stablished. For example, indomethacin, much more potent antiinflammatory agent than flufenamic acid, is less potent as PDE inhibitor. Aspirin, with relatively good antiinflammatory activity, has by far the highest I_{50} among all the compounds tested.

Discussion

The results show that several steroidal antiinflammatory drugs are inhibitors of beef heart PDE. This property has already been described for some of these compounds. The I_{50} values obtained in this work are in agreement with those reported in the literature (8, 11). This PDE inhibition might increase the intracellular cyclic AMP levels and it has been suggested that this fact could contribute to the antiinflammatory activity of these compounds (5, 6, 13). However, as already pointed out by other authors, the drug concentrations that produce 50% inhibition of PDE activity are much higher than the plasma concentrations cur-

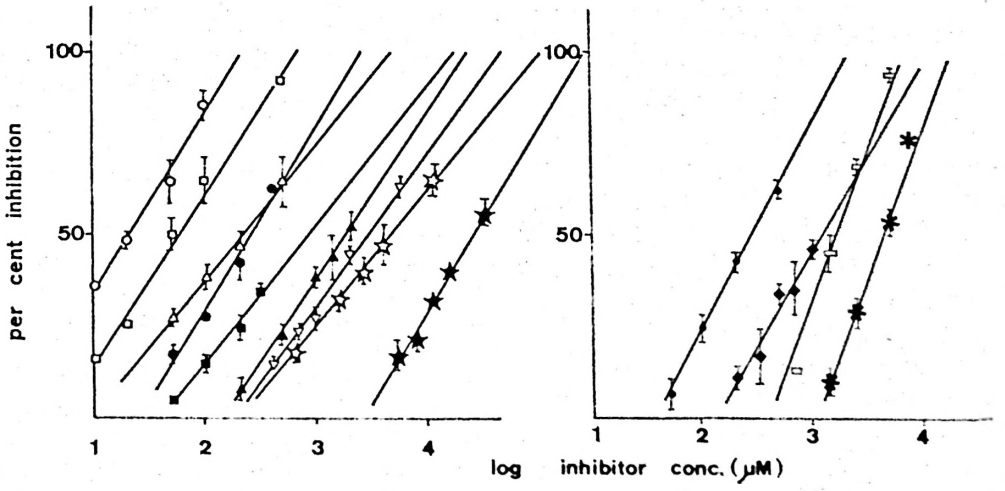


Fig. 2. *Inhibition of beef heart phosphodiesterase by aspirin (★), diclofenac sodium (●), flufenamic acid (○), ibuprofen (▽), indomethacin (□), ketoprofen (◆), mefenamic acid (△), naproxen (▲), UR-333 (●), UR-337 (■), UR-1501 (☆), UR-1522 (✳), UR-1524 (□).*
Ordinate: inhibition of enzyme activity in percent of control. Lines represent the best fit, as determined by linear least-squares regression analysis. Vertical bars represent standard error o the mean when three or more determinations were averaged.

Table I. *Inhibition of the beef heart phosphodiesterase (PDE) and Antiinflammatory activity of various antiinflammatory substances.*

Drug	PDE inhibition I_{50} (μM)*	Antiinflammatory activity	
		Carragenin edema in the rat** I_{50} (mg/kg)	Clinical daily dose in rheumatoid arthritis (mg/day)***
Flufenamic acid	26	38.8	400-600
Indomethacine	57	1.2	75-150
Mefenamic acid	215	93.3	1,500
Diclofenac sodium	255	1.6	75
UR-333	285	341.9	—
UR-337	900	>341.9	—
Ketoprofen	1,190	4.1	100-300
UR-1524	1,630	—	—
Naproxen	1,940	30.8	300-500
Ibuprofen	2,850	—	600-1,600
UR-1522	4,500	—	—
UR-1501	5,400	92.5	—
Aspirin	26,000	128.2	2,500-6,000

* Micromolar concentrations inducing a 50 per cent inhibition of the beef heart phosphodiesterase calculated by least-squares regresion analysis from the values of figure 2.
** Oral dose which produces a 50 per cent inhibition of the carragenin induced edema in the rat paw.
*** From Dürrigh et al. (4); Lombardino et al. (9) and Lwoff and Simon (10).

rently obtained in the clinical use of these compounds (5, 14). Moreover, this study shows a lack of correlation between the order of potency of several antiinflammatory agents and their order of potency as PDE inhibitors. For instance, UR-333 has a very weak antiinflammatory activity, but its I_{50} value is similar to that of diclofenac sodium, a very powerful antiinflammatory agent.

Other oustanding examples are aspirin and indomethacin. Aspirin has a moderate antiinflammatory activity but its PDE inhibitory activity is negligible. On the other hand, indomethacin which is the most potent antiinflammatory studied, has an I_{50} greater than flufenamic acid.

These results do not completely rule out the possibility that cyclic AMP plays an important rôle in the inflammatory response. One can imagine, for instance, that another cellular PDE more closely linked to the inflammatory response could have an important function. However, it can be clearly concluded from the present study, that the measurement of the inhibition of beef heart PDE produced by potential antiinflammatory agents can not be used as a test to predict their antiinflammatory activity.

Resumen

Se estudia el efecto inhibidor de diversos antiinflamatorios no esteroidales sobre la actividad de la AMPc-fosfodiesterasa (PDE) de corazón de buey. La actividad enzimática se determinó según el método radioactivo de Thompson y Appleman, modificado, utilizando como sustrato H^3-AMPc. Los resultados obtenidos, aunque no excluyen la posible participación del AMPc en los procesos inflamatorios, demuestran que no existe correlación entre la inhibición de la PDE de corazón de buey y la actividad antiinflamatoria determinada por el método de la carragenina en la rata (Winter). Se concluye, en consecuencia, que la inhibición de la PDE de corazón de buey por diversas drogas no puede utilizarse como criterio válido para predecir su actividad antiinflamatoria.

References

1. BERTELLI, A.: *J. Dent. Res.*, **51**, 235-243, 1972.

2. Di Rosa, M., Giroud, J. P. and Willoughby, D. A.: *J. Path. Bac.*, **104**, 15-17, 1971.
3. Di Rosa, M., Papadimitriou, J. M. and Willoughby, D. A.: *J. Path. Bact.*, **105**, 239-254, 1971.
4. Dürrigl, T., Vitans, M., Pucar, I. and Miko, M.: *J. Int. Med. Res.*, **3**, 139-144, 1975.
5. Flower, R., Gryglewski, R., Herbaczynska-Cedro, K. and Vane, J. R.: *Nature New Biol.*, **238**, 104-106, 1972.
6. Ichikawa, A., Nagasaki, M., Umezu, K., Hayashi, H. and Tomita, K.: *Biochem. Pharm.*, **21**, 2615-2626, 1972.
7. Ignarro, J. L. and Colombo, C.: *Nature New Biol.*, **239**, 155-156, 1972.
8. Ishikawa, Y. and Newcombe, D. I.: *Proc. Soc. exp. Biol. Med.*, **152**, 105-110, 1976.
9. Lombardino, J. G., Otternes, I. G. and Wiseman, E. H.: *Arzneim. Forsch.*, **25**, 1629-1634, 1975.
10. Lwoff, J. M. and Simon, P.: *Thérapeutique*, **25**, 345-351, 1975.
11. Stefanovich, V.: *Res. Comm. Path. Pharmacol.*, **7**, 573-582, 1974.
12. Thompson, J. and Appleman, M. A.: *Biochemistry*, **10**, 311-316, 1971.
13. Tomita, K., Ichikawa, A. and Hayashi, H.: *Jap. J. Pharmacol.*, **22**, 8, 1972.
14. Tomlinson, R. V., Ringold, H. J., Qureshi, M. C. and Forchielli, E.: *Biochem. Biophys. Res. Comm.*, **46**, 552-559, 1972.
15. Vane, J. R.: *Nature New Biol.*, **231**, 232-235, 1971.
16. Weinryb, I., Chasin, M., Free, C. A., Harris, D. N., Goldenberg, H., Michel, I. M., Raik, V. S., Philips, M., Samaniego, S. and Hess, S. M.: *J. Pharm. Science*, **61**, 1556-1566, 1972.
17. Whitehouse, M. W. and Haslam, J. M.: *Nature* **196**, 1323-1324, 1962.
18. Winter, C. A., Risley, W. A. and Nuss, G. W.: *Proc. Soc. Exp. Biol. Med.*, **111**, 544,547, 1962.