Biochemical Pharmacology, Vol. 42, No. 8, pp. 1633–1634, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

SHORT COMMUNICATIONS

Possible mechanism of action of desferrioxamine and 2,2′-bipyridyl on inhibiting the *in vitro* growth of *Plasmodium falciparum* (FCR 3 strain)

(*Received* 18 *April* 1991; *accepted* 10 *June* 1991)

The influence of iron on the outcome of various infections has been noted previously [1]. Clinical observations have suggested that iron deficiency may be protective against malaria [2]. Various researchers have shown that certain iron chelators block the proliferation of *Plasmodium falciparum in vitro* and *in vivo* [3, 4]. However the exact mode of malaria inhibition by iron chelators remains undefined. The effects of desferrioxamine (DFO) and 2,2′-bipyridyl individually and in combination on the *in vitro* survival of the *P. falciparum* FCR 3 strain was studied, in order to further elucidate possible mechanisms of action.

Materials and Methods

P. falciparum (FCR 3 strain) was cultured *in vitro* according to the method described by Freese *et al.* [5]. The parasites were synchronized using the method of Lambros and Vanderberg [6]. Iron chelators used were desferrioxamine mesylate (Ciba Geigy) and 2,2′-bipyridyl (Fluka). Each concentration and combination of chelators was analysed in quadruplicate. The synchronized parasites were added to the wells predosed with the chelators and incubated at 37° for 24 hr. After 24 hr the parasites were labelled with [^{3}H]hypoxanthine (Amersham) and incubated for a further 24 hr. Incorporation of [^{3}H]hypoxanthine was used as an index of growth and the degree of parasitaemia [7]. The chelator sensitivity studies were performed in 96-well microtiter plates (Sterilin Ltd). The parasites were harvested with an automatic cell harvester (Flow Lab.). The dose–response curves of DFO and 2,2′-bipyridyl alone and in combination were determined. The combined effect of DFO and iron-saturated 2,2′-bipyridyl was also evaluated using ferrous ammonium sulphate at (1:1) molar ratio. Results were expressed as a percentage of controls with no chelator added.

Results and Discussion

Previous studies have suggested alternative mechanisms of action of iron chelation in malaria [3, 8]. In our study, the IC_{50} (±SE) values of DFO and 2,2′-bipyridyl alone were 5.15 (±0.29) μM and 12.36 (±0.50) μM, respectively. Both DFO and 2,2′-bipyridyl were shown to inhibit independently the growth of *P. falciparum* (FCR 3) *in vitro*. If both chelators had inhibited the growth of the parasites by simply withholding iron, then the combined effect of the two would be either additive or synergistic. 2,2′-Bipyridyl at various concentrations when combined with DFO (2 μM) showed a relative increase in parasitaemia compared to the 2,2′-bipyridyl alone, thus suggesting that these concentrations of 2,2′-bipyridyl antagonized the antimalarial effect of DFO. This antagonistic effect is less pronounced with higher DFO concentrations (Table 1). To exclude the possibility of a direct interaction of DFO with 2,2′-bipyridyl changes in the UV spectrum of 2,2′-bipyridyl were measured *in vitro* after addition of DFO. There was no change in the spectra obtained. To confirm that 2,2′-bipyridyl antagonized the antimalarial effect of DFO noted above, the iron chelating effect of the former was eliminated by saturating it with ferrous ammonium sulphate at equimolar concentration. When 6 μM DFO (approximate value of IC_{50}) was combined with various concentrations of Fe^{2+} saturated 2,2′-bipyridyl (0–20 μM), the resultant dose–response curve was a straight line (slope was not significantly different from 0) reflecting the effect of DFO alone. The effect of 2,2′-bipyridyl with and without Fe^{2+} saturation, on DFO, is presented in Fig. 1. All the data for the effect of non-iron saturated 2,2′-bipyridyl were fitted using Enzfitter Software (Elsevier Biosoft) using the following equation:

$$\text{Effect}\,(\%) = \frac{W_1}{1+\left(\frac{C}{C_{50_1}}\right)^{P_1}} + \frac{W_2}{1+\left(\frac{C}{C_{50_2}}\right)^{P_2}}$$

Table 1. The combined effect of desferrioxamine and 2,2′-bipyridyl on the survival of the parasites

Concentration of 2,2′-bipyridyl (μM)	Concentration of desferrioxamine (μM)		
	0	2	12
0	100	87.2 ± 8.85	14.9 ± 2.82
6	75.0 ± 8.22	96.1 ± 3.08	22.3 ± 0.79
8	69.2 ± 1.25	86.8 ± 6.41	25.3 ± 3.90
10	63.7 ± 9.42	95.0 ± 4.02	19.8 ± 2.66
12	58.2 ± 2.31	79.6 ± 4.56	18.1 ± 2.68
14	42.4 ± 6.72	78.5 ± 4.44	14.3 ± 1.99
18	37.5 ± 7.69	56.6 ± 4.55	9.5 ± 2.56

The [^{3}H]hypoxanthine incorporation is represented as a percentage of the control. Each reading is stated with standard deviation and is a mean of at least three readings.

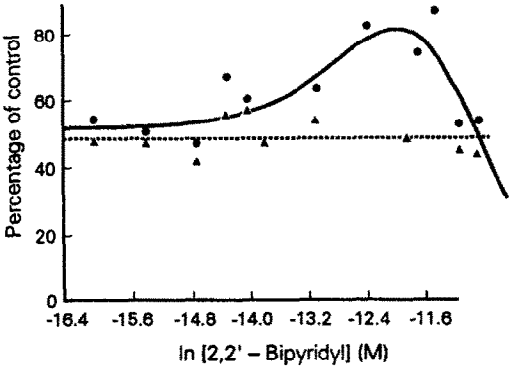

Fig. 1. The sensitivity of *P. falciparum* malaria parasites to the combined effect of desferrioxamine and 2,2′-bipyridyl. [^{3}H]Hypoxanthine incorporation (performed at least in triplicate) is represented as a percentage of controls. Key: (●—●) represents the combined effect of a constant concentration of DFO (6 μM) with different concentrations of 2,2′-bipyridyl; (▲---▲) represents the combined effect of DFO (6 μM) and equimolar ratios of Fe^{2+} and 2,2′-bipyridyl.

which represents additive or antagonistic effects of two sigmoid dose–response curves. The parameters estimated by the equation were as follows: $C_{50_1} = 15.8\,\mu M$; $C_{50_2} = 4.9\,\mu M$; $P_1 = 1.9$; $P_2 = 1.5$; $W_1 = 136.5\%$; $W_2 = -84.5\%$ where W_1 and W_2 represent the maximal effect achieved; C represents the concentration of drug; C_{50_1} and C_{50_2} represent the IC_{50} of the drug; and P_1 and P_2 represent the slope of the dose–response curve.

The W_2 parameter is negative indicating an antagonistic effect. The contribution of the second term in the equation on the overall effect becomes less pronounced with increasing concentration of 2,2′-bipyridyl.

In summary it can be concluded from the finding that non-iron saturated 2,2′-bipyridyl combined with desferrioxamine resulted in an antagonistic effect that other mechanisms of action of iron chelators besides iron deprivation inhibit the growth of the parasites.

Department of Experimental and Clinical Pharmacology
7 York Road
Parktown, 2193
Johannesburg
South Africa

K. T. JAIRAM
I. HAVLIK
F. S. E. MONTEAGUDO*

* Correspondence to Prof. F. S. E. Monteagudo.

REFERENCES

1. Peto TEA and Hershko C, Iron and infection. *Clin Haematol* **2**: 435–458, 1989.
2. Smith AW, Hendrickse RG, Harrison C, Hayes RJ and Greenwood BM, The effects on malaria of treatment of iron-deficiency anaemia with oral iron in Gambian children. *Ann Trop Pediatr* **9**: 17–23, 1989.
3. Iheanacho EN, Samuni A, Avramovici-Grisaru S, Sarel S and Spira DT, Inhibition of *Plasmodium falciparum* growth by a synthetic iron chelator. *Trans R Soc Trop Med Hyg* **84**: 213–216, 1990.
4. Hershko C and Peto TEA, Desferrioxamine inhibition of malaria is independent of host iron status. *J Exp Med* **168**: 375–387, 1988.
5. Freese JA, Sharp BL, Ridle FL and Markus MB, *In vitro* culturation of Southern Africa strains of *Plasmodium falciparum* and gametocytogenesis. *S Afr Med J* **73**: 720–722, 1988.
6. Lambros C and Vanderberg JP, Synchronization of *Plasmodium falciparum* erythrocytic stages in culture. *J Parasitol* **65**: 418–420, 1979.
7. Desjardins RE, Canfield CJ, Haynes JD and Chulay JD, Quantitative assessment of antimalarial activity *in vitro* by a semi automated microdilution technique. *Antimicrobial Agents Chemother* **16**: 710–718, 1979.
8. Klebanoff SJ, Waltersdorph AM, Michel BR and Rosen H, Oxygen based free radical generation by ferrous irons and desferrioxamine. *J Biol Chem* **264**: 19765–19771, 1989.

Biochemical Pharmacology, Vol. 42, No. 8, pp. 1634–1637, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

Effects of glimepiride and glibenclamide on insulin and glucagon secretion by the perfused rat pancreas

(*Received* 5 *April* 1991; *accepted* 8 *June* 1991)

Hypoglycemic sulfonylureas stimulate insulin release. Conflicting data were reported, however, on their direct effect upon glucagon release (see Ref. 1 for review). Moreover, various sulfonylureas may differ from one another in terms of their glucagonotropic effect [2]. The present study aims at comparing the effects of glimepiride and glibenclamide upon both insulin and glucagon secretion.

Materials and Methods

The present study was conducted on 20 fed female Wistar rats (body weight: 244 ± 3 g). The perfusion of the pancreas [3, 4] and measurement of insulin and glucagon output [5, 6] were performed as previously described. The flow rate averaged 1.36 ± 0.03 mL/min and the perfusion pressure 30 ± 1 mmHg. The glucose concentration of the perfusate was increased from 5.6 to 16.7 mM at the 86th min. Glimepiride and glibenclamide (final concentration 0.1 μM) were first dissolved in dimethyl sulfoxide (DMSO) (final concentration 0.01%, v/v) and administered from the 41st to 60th min. After perfusion, the weight of the pancreas averaged 634 ± 29 mg, its insulin content 183 ± 15 μg/g and its glucagon content 10 ± 1 μg/g (N = 20 in all cases). All results are expressed as the mean value ± SEM. Statistical comparisons were conducted using the two-tailed non-paired *t*-test.

Results

Both glimepiride and glibenclamide caused a biphasic increase of insulin release, which between the 55th and 60th min was about five times higher than basal output (Fig. 1). The stimulation of insulin release was not reversed upon the removal of the sulfonylureas. The later rise in D-glucose concentration provoked a marked and biphasic increase in insulin output. Between the 87th and 100th min, the output of insulin was higher ($P < 0.01$) in the pancreases first exposed to glimepiride or glibenclamide than in the control experiments. The glucose-induced increment in insulin output (min 87–100 vs min 65–85) was not significantly higher, however, in the glimepiride group (+ 46.3 ± 4.3 ng/min) or glibenclamide group (+ 50.6 ± 8.1 ng/min) than in the control group (+ 34.8 ± 3.2 ng/min).

The infusion of the sulfonylureas induced a small but significant fall in glucagon output (Fig. 2). Thus, the paired