The antiarrhythmic activities of 6′-hydroxycinchonine, 6′-benzyloxycinchonine and 6′-allyloxycinchonine compared with quinidine in mice

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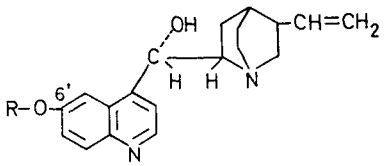
Several attempts have been made to discover new and safer quinidine-like antiarrhythmic agents (Van Dongen et al 1937, 1938, 1950; Christensen et al 1970) but no advantages over quinidine were observed. The synthesis of 6′-hydroxycinchonine as a potential antiarrhythmic agent was achieved recently in our laboraories by Small and Rosenberg (unpublished) by substituting the 6′-methoxy group of quinidine with a hydroxy group. Using aconitine as the arrhythmogenic agent, the antiarrhythmic activities of 6′-hydroxycinchonine as well as the 6′-benzyloxy- and 6′-allyloxy-derivatives were compared with the antiarrhythmic efficacy of quinidine. LD50 and ED50 values were compared for quinidine and 6′-hydroxycinchonine using this technique.



Compounds	R
Quinidine	$-CH_3$
6′-Hydroxycinchonine	$-H$
6′-Allyloxycinchonine	$-CH_2{=}CH{-}CH_3$
6′-Benzyloxycinchonine	$-CH_2-C_6H_5$

Male mice (19–25 g) of Swiss Webster strain, Sasco Laboratories, Omaha, Nebraska were anaesthetized with sodium pentobarbitone (50 mg kg^{-1} i.p.). Each mouse then received a predetermined dose of the test compound or the 0·9% w/v NaCl (saline) vehicle by tail vein infusion; 3 min was allowed before induction of arrhythmias. Groups of 10 mice were pretreated for about 3 min with either the saline vehicle, quinidine sulphate, 6′-hydroxycinchonine hydrochloride, 6′-benzyloxycinchonine hydrochloride or 6′-allyloxycinchonine hydrochloride (30 mg kg^{-1}, i.v. in 0·9% NaCl). The dose employed was based on preliminary investigations.

Arrhythmias were induced with aconitine, according to Dadkar & Bhattacharya (1974) as modified by Nwangwu et al (1977). A tail vein was cannulated for continuous intravenous infusion using a Sage Instrument Model 355 syringe pump. Aconitine (5 $\mu g\ ml^{-1}$), prepared just before use, was delivered at 0·25 ml min^{-1} and elicited cardiac arrhythmias in 100% of the animals in the control group.

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Table 1. ED50 and LD50 values for quinidine and 6′-hydroxycinchonine in mice.

	ED50 (mg kg^{-1})	LD50 (mg kg^{-1})
Quinidine	22·4 (19·5–25·8)	202·0 (178·8–228·3)
6′-Hydroxycinchonine hydrochloride	23·5 (20·6–26·8)	304·0 (249·2–370·9)

For both the ED50 and LD50 determinations, groups of 10 mice were given five equally spaced logarithmic doses of either quinidine or 6′-hydroxycinchonine. The time to onset of aconitine-induced arrhythmias were recorded as the endpoint for each animal for the ED50 determinations with the drugs being given intravenously. Death within 24 h constituted a positive response for the LD50 determinations, with the drugs being given intraperitoneally. The ED50 and LD50 values were obtained from the respective probit of response versus dose plots. The values in parentheses are the 95% confidence intervals as determined by the method of Litchfield & Wilcoxon (1949).

Arrhythmias were measured by e.c.g. (lead II) recordings using a physiograph (Type PMP-4A, E & M Instrument Co., Inc.) (Nwangwu et al 1977). Endpoints were the time to onset of initial arrhythmia, i.e. the time at which the first discernible sign of persistent (>5 s) deviation from normal rhythm was observed and the onset of ventricular tachycardia, i.e. the time at which persistent (>5 s) ventricular tachycardia occurred. The endpoints for all pretreatment groups were compared for significant differences using the Student's *t*-test.

To assess the ED50 (doses at which 50% of the mice displayed positive antiarrhythmic activity), groups of 10 animals were pretreated with 6′-hydroxycinchonine hydrochloride, quinidine sulphate or vehicle, with equally spaced logarithmic doses determined in pilot studies. The time to onset of aconitine-induced initial arrhythmia for each mouse was recorded as the endpoint. The times were compared with the mean and 2 standard errors (mean ± 2 s.e.) for the saline group and a greater value was taken as a positive response. Plots of per cent response versus the log dose were used to produce dose-response curves for both drugs. The per cent responses were converted to probit of responses and dose-probit curves were generated from which ED50 values were obtained. The results are in Table 1. The 95% confidence limits were determined by the method of Litchfield & Wilcoxon (1949).

Table 2. Prolongation of onset-time to aconitine-induced initial arrhythmia and ventricular tachycardia in mice by quinidine and 6′-hydroxycinchonine. Mice were pretreated with the saline vehicle, quinidine or 6′-hydroxycinchonine, and arrhythmias were induced by the slow intravenous infusion of aconitine. The time (s) to each of the two endpoints was recorded. Each value represents the mean (±s.e.).

Pretreatment	Onset of initial arrhythmia (s)	Onset of ventricular tachycardia (s)
Control 0·9% w/v NaCl	153 ± 5	194 ± 11
Quinidine sulphate (30 mg kg^{-1} i.v.)	194 ± 11[a]	294 ± 44[b]
6′-Hydroxycinchonine hydrochloride (30 mg kg^{-1} i.v.)	198 ± 8[c,d]	251 ± 22[b,d]

[a] $P < 0.01$ with respect to control group. [b] $P < 0.05$ with respect to control group. [c] $P < 0.01$ with respect to control group. [d] No significant difference with respect to quinidine group.

For the LD50, groups of 10 mice were treated as for the ED50 with equally spaced logarithmic doses previously determined. Each group was housed in a separate cage and observed after 24 h. Death within the 24 h constituted a positive response. No attempt was made to ascertain cause of death. Dose response and dose-probit curves were based upon the number of deaths at each dose. The LD50 values (Table 1) were obtained from the dose-probit curves.

Table 3. Prolongation of onset-time to aconitine-induced initial arrhythmia and ventricular tachycardia in mice by 6′-allyloxycinchonine and 6′benzyloxycinchonine.

Pretreatment	Onset of initial arrhythmia (s)	Onset of ventricular tachycardia (s)
Control 0·9% w/v NaCl	160 ± 5	186 ± 6
6′-Benzyloxycinchonine hydrochloride (30 mg kg^{-1}, i.v.)	195 ± 8[a]	217 ± 6[c]
6′-Allyloxycinchonine hydrochloride (30 mg kg^{-1}, i.v.)	215 ± 9[b,c]	261 ± 11[b,d]

Mice were pretreated with vehicle, or drug, and arrhythmias were induced by the slow intravenous infusion of aconitine. The time (s) to each of the two endpoints was recorded. Each value represents the mean (±s.e.) of 10 mice. [a] $P < 0.05$ with respect to control group. [b] $P < 0.001$ with regard to control group. [c] N.S. with respect to 6′-benzyloxycinchonine group. [d] $P < 0.001$ with respect to 6′-benzyloxycinchonine group.

Pretreatment of mice with either quinidine or 6′-hydroxycinchonine at 30 mg kg^{-1} significantly prolonged the time to onset of aconitine-induced arrhythmias and ventricular tachycardia. (Table 2). There was no significant difference between endpoints for the quinidine and 6′-hydroxycinchonine.

The ability of 6′-benzyloxycinchonine to prolong the onset of aconitine-induced arrhythmia was similar to that observed for quinidine and 6′-hydroxycinchonine. However, its ability to prolong onset of aconitine-induced ventricular tachycardia was markedly lower than quinidine. 6′-Allyloxycinchonine behaved similarly to quinidine and 6′-hydroxycinchonine (Table 3).

An ED50 value of approximately 23 mg kg^{-1}, i.v., was obtained for quinidine and 6′-hydroxycinchonine (Table 3). The LD50 values (also in Table 1) show the LD50 for 6′-hydroxycinchonine is approximately 50% greater than that for quinidine.

Thus, 6′-hydroxycinchonine, 6′-allyloxycinchonine and 6′benzyloxycinchonine have been shown to possess significant antiarrhythmic activity by previously verified methodology (Nwangwu et al 1977). 6′Hydroxycinchonine and 6′allyloxycinchonine were as effective as quinidine in prolonging the onset of both aconitine-induced arrhythmia and ventricular tachycardia. 6′-Benzyloxycinchonine was less active in its ability to prolong onset of ventricular tachycardia.

While the ED50 values for quinidine and 6′-hydroxycinchonine were similar, the LD50 for quinidine was lower (202 mg kg^{-1}) than that for 6′-hydroxycinchonine (304 mg kg^{-1}) and compares with values of 190 mg kg^{-1} reported by Calesnick et al (1951), and 245 ± 21 mg kg^{-1} by Mokler & Van Arman (1962).

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