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Stereoselective Effects of (R)- and (S)-Carvedilol in Humans

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ABSTRACT Carvedilol is currently used as the racemic mixture, (R,S)-carvedilol, consisting of equal amounts of (R)-carvedilol, an alpha-blocker, and (S)-carvedilol, an alpha- and beta-blocker, which have never been tested in their optically pure forms in human subjects. We performed a randomized, double-blind, placebo-controlled, cross-over study in 12 healthy male volunteers. Subjects received single oral doses of 25 mg (R,S)-carvedilol, 12.5 mg (R)-carvedilol, 12.5 mg (S)-carvedilol, and placebo at 8 AM as well as at 8 PM. Exercise was performed at 11 AM, and heart rate and blood pressure were measured at rest and after 10 min of exercise. Urine was collected between 10 AM and 6 PM, as well as between 10 PM and 6 AM, and the amounts of urinary 6-hydroxy-melatonin sulfate (aMT6s) were determined by RIA. Compared to placebo, (R)-carvedilol increased heart rate during exercise (+4%, $P < 0.05$) and recovery (+10%, $P < 0.05$); (S)-carvedilol decreased heart rate during exercise (–14%, $P < 0.05$) and recovery (–6%, $P < 0.05$), and systolic blood pressure during exercise (–12%, $P < 0.05$); (R,S)-carvedilol decreased heart rate during exercise (–11%, $P < 0.05$), and systolic blood pressure at rest (–7%, $P < 0.05$) and during exercise (–10%, $P < 0.05$). None of the agents had any significant effect on the release of aMT6s. Our results indicate that only (S)-carvedilol causes beta-blockade, whereas (R)-carvedilol appears to increase sympathetic tone, presumably as a physiological reaction to the decrease of blood pressure caused by alpha-blockade. None of the drugs had any influence on melatonin release. The weak clinical net effect of beta-blockade of (R,S)-carvedilol at rest might be one reason why this drug causes fewer side effects than other beta-blockers, such as a reduction of nocturnal melatonin release. *Chirality 13:342–346, 2001.*

KEY WORDS: beta-blockers; alpha-blockers; heart rate; melatonin; chirality

Carvedilol is known as an antagonist of adrenergic alpha- and beta-receptors.[1–4] It is marketed and used as the racemic mixture, (R,S)-carvedilol. However, it has been shown in vitro that the affinity of (S)-carvedilol to adrenergic beta-receptors is about 100 times greater than that of the respective (R)-enantiomer, whereas both (R)- and (S)-carvedilol inhibit adrenergic alpha-receptors to the same extent.[5,6]

Recently, a study in healthy volunteers using the optically pure (R)- and (S)-enantiomers and the racemic mixtures of propranolol and atenolol revealed that half-dosed (S)-propranolol and (S)-atenolol decrease heart rate and systolic blood pressure both at rest and during exercise to the same extent as the currently used (R,S)-propranolol and (R,S)-atenolol, whereas the respective (R)-enantiomers lack this effect.[7] So far, similar data are not available for carvedilol.

More recently, we showed that (S)-propranolol and (S)-atenolol markedly decrease nocturnal melatonin release in healthy subjects (–80% and –86%, respectively), whereas (R)-propranolol and (R)-atenolol do not.[8] This finding was explained by the fact that the synthesis of melatonin in and its release from the pineal gland are stimulated by norepinephrine via sympathetic $beta_1$-receptors, and this process is further potentiated by stimulation of $alpha_1$-receptors.[9–15] However, there was a quite unexpected and unexplained finding in this study,[8] namely, that (R,S)-carvedilol, which blocks both alpha- and beta-adrenoceptors, had no influence on nocturnal melatonin release, a result that deserves further investigation.

In addition, a study in patients suffering from congestive heart failure showed that long-term therapy with (R,S)-metoprolol caused an increase ("upregulation") of beta-adrenoceptor density,[16] a finding usually observed in patients on beta-blockers without intrinsic sympathomimetic activity. However, there was no upregulation of beta-

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receptor density with (R,S)-carvedilol.[16] This finding also remained unexplained and merits further investigation.

The present randomized, double-blind, placebo-controlled, crossover study was performed in order to address these unresolved issues. Twelve healthy male volunteers received single oral doses of 25 mg (R,S)-carvedilol, 12.5 mg (R)-carvedilol, 12.5 mg (S)-carvedilol, and placebo at 8 AM and 8 PM. Exercise was performed at 11 AM and heart rate and blood pressure were measured at rest, during exercise, and after 15 min of recovery in order to determine clinically relevant alpha- and beta-blockade.

Furthermore, urine was collected during the daytime (10 AM to 6 PM) and at night (10 PM to 6 AM), and urinary 6-hydroxy-melatonin sulfate (aMT6s, the main metabolite of melatonin, which is almost completely eliminated in urine) was determined in order to investigate the influence of the stereoisomers of carvedilol on daytime and nocturnal melatonin release.

MATERIALS AND METHODS

Study Protocol

Twelve healthy male volunteers (all of the CYP2D6 extensive metabolizer phenotype) were included in the study. They entered the laboratory at 7:30 AM following an overnight fast and remained fasted until the end of the first examination at 11:30 AM. All beverages containing caffeine or alcohol were strictly forbidden between 8 PM the day before until 6 AM the day after. At intervals of 1 week, subjects received single oral doses of 25 mg (R,S)-carvedilol, 12.5 mg (R)-carvedilol, 12.5 mg (S)-carvedilol, and placebo at 8 AM and 8 PM according to a randomized, double-blind, placebo-controlled, crossover protocol. After adaptation of subjects to the apparatus, heart rate (by continuous ECG monitoring) and blood pressure (by sphygmomanometry) were measured at rest in a sitting position at 11 AM, then exercise (80% of mean individual work load) was performed over 10 min using a bicycle ergometer. Heart rate and blood pressure were determined during the last minute of exercise, then subjects remained rested for 15 min in a supine position. Heart rate and blood pressure were measured again during the last minute of this period of recovery.

Total urine was collected between 10 AM and 6 PM as well as between 10 PM and 6 AM and quality of sleep was assessed with a questionnaire.

The investigation described above was repeated at intervals of 7 days with 25 mg (R,S)-carvedilol, 12.5 mg (R)-carvedilol, 12.5 mg (S)-carvedilol, and placebo given according to a randomized, double-blind, crossover protocol. All investigations were performed according to the current laws of Austria and the study was approved by the Ethics Committee of the Medical Faculty of the Karl Franzens University, Graz, Austria

Materials

(R,S)-carvedilol (an exact 50:50 mixture of (R)- and (S)-carvedilol), optically pure (R)-carvedilol, and optically pure (S)-carvedilol were supplied by Hoffmann-La Roche (Basel, Switzerland). The pharmaceutical formulations (hard gelatin capsules) containing 25 mg (R,S)-carvedilol, 12.5 mg (R)-carvedilol, 12.5 mg (S)-carvedilol, or placebo, together with mannitol and carbosil as auxiliary materials, were prepared according to the specifications of the European Pharmacopoeia at the Institute of Pharmaceutical Technology, Karl Franzens University, Graz, Austria.

Analysis of 6-Hydroxy-melatonin Sulfate (aMT6s) in Urine

Urinary aMT6s was determined according to the method of Arendt et al.[17] with a commercially available RIA kit (Stockgrand, Guildford, UK) using an iodinated tracer. The intra- and interassay coefficients of variation were 4% and 7%, respectively.

Data Analysis

Results are given as arithmetic means ± 1 SD unless otherwise indicated. Significances of differences were calculated by repeated measures ANOVA (Friedman's Repeated Measures ANOVA on Ranks when applicable) and Student-Newman-Keuls test was used for posthoc testing. A P-value < 0.05 was considered statistically significant.

RESULTS

Effects of the drugs on hemodynamic parameters and aMT6s in urine are listed in Table 1, effects on heart rate are displayed in Figure 1. Compared to placebo, (R)-carvedilol increased heart rate during exercise (+4 ± 2%, $P < 0.05$) and recovery (+10 ± 3%, $P < 0.05$) with no effect on any of the other parameters. (S)-carvedilol decreased heart rate during exercise (−14 ± 4%, $P < 0.05$) and systolic blood pressure during exercise (−12 ± 3%, $P < 0.05$) with no effect on any of the other parameters. (R,S)-carvedilol decreased heart rate during exercise (−11 ± 3%, $P < 0.05$), and systolic blood pressure at rest (−7 ± 3%, $P < 0.05$) and during exercise (−10 ± 3%, $P < 0.05$) with no effect on any of the other parameters. None of the drugs significantly affected daytime or nocturnal release of aMT6s or quality of sleep.

DISCUSSION

Our findings indicate that currently used (R,S)-carvedilol and half the dose optically pure (S)-carvedilol decrease heart rate during exercise to the same extent, thus indicating that only (S)-, but not (R)-, carvedilol causes beta-blockade in humans when the drugs are given in clinical doses. These data obtained in healthy subjects are in good accordance with results from in vitro studies showing that the beta-blocking potency of (S)-carvedilol is about 100-fold greater than that of (R)-carvedilol.[5,6] In addition, the results with (R)-, (S)-, and (R,S)-carvedilol in the present study correspond well with those found with (R)-, (S)-, and (R,S)-propranolol, as well as with those obtained with (R)-, (S)-, and (R,S)-atenolol in a human study using a similar protocol.[7] However, the reduction of heart rate during exercise in the present study caused by 25 mg (R,S)-carvedilol and 12.5 mg (S)-carvedilol (−11% and −14%, respectively) appeared somewhat lower than that obtained with 80 mg (R,S)-propranolol and 40 mg (S)-propranolol (−19% in both cases) and 100 mg (R,S)-atenolol and 50 mg (S)-atenolol (−23% in both cases).[7] Thus, clinically recommended doses of (R,S)-carvedilol appear to cause some-

TABLE 1. Results obtained 3 h after oral administration of placebo, 12.5 mg (R)-carvedilol, 12.5 mg (S)-carvedilol, and 25 mg (R,S)-carvedilol

	Placebo	12.5 mg (R)-carvedilol	12.5 mg (S)-carvedilol	25 mg (R,S)-carvedilol
Heart rate at rest (n.s.)	56 ± 8	61 ± 7	56 ± 11	56 ± 8
		n.s.	n.s.	n.s.
Systolic BP at rest (p = 0.002)	116 ± 10	114 ± 6	113 ± 9	108 ± 8
		n.s.	n.s.	p < 0.05
Diastolic BP at rest (n.s.)	78 ± 6	77 ± 5	78 ± 8	75 ± 6
		n.s.	n.s.	n.s.
Heart rate during exercise (p < 0.001)	143 ± 14	149 ± 16	124 ± 12	127 ± 14
		p < 0.05	p < 0.05	p < 0.05
Systolic BP during exercise (p < 0.001)	172 ± 23	169 ± 24	152 ± 22	155 ± 23
		n.s.	p < 0.05	p < 0.05
Diastolic BP during exercise (n.s.)	71 ± 7	68 ± 7	67 ± 7	67 ± 5
		n.s.	n.s.	n.s.
Heart rate during recovery (p < 0.001)	72 ± 7	78 ± 9	67 ± 11	71 ± 10
		p < 0.05	n.s.	n.s.
Systolic BP during recovery (p = 0.005)	113 ± 7	112 ± 6	108 ± 6	107 ± 4
		n.s.	p < 0.05	p < 0.05
Diastolic BP during recovery (p = 0.015)	78 ± 5	79 ± 5	75 ± 5	75 ± 6
		n.s.	n.s.	n.s.
aMT6s in daytime urine (n.s.)	4.3 ± 1.8	3.8 ± 1.4	3.2 ± 1.7	4.1 ± 1.9
		n.s.	n.s.	n.s.
aMT6s in nocturnal urine (n.s.)	29 ± 16	36 ± 34	27 ± 19	25 ± 14
		n.s.	n.s.	n.s.

Heart rate, beats/min; systolic and diastolic blood pressure (BP), mmHg.
Total amounts of 6-sulfatoxy melatonin (aMT6s) (μg) in daytime (10 AM–6 PM) and nocturnal urine (10 PM–6 AM).
Means ± 1 SD; n = 12. Significances of differences within groups were calculated by repeated measures ANOVA (Friedman's Repeated Measures ANOVA on Ranks when applicable) and post-hoc analyses from placebo by Student-Newman-Keuls test.

what weaker clinical effects resulting from blockade of beta-adrenoceptors than those of (R,S)-propranolol and (R,S)-atenolol. However, it has to be emphasized that this finding refers only to the pure beta-blocking effects, but not the overall effects, of these drugs. The weaker beta-blocking effects of (R,S)-carvedilol might also be the reason for the lesser side effects resulting from beta-blockade despite the fact that (R,S)-carvedilol is a nonselective beta-adrenergic antagonist.[1–4]

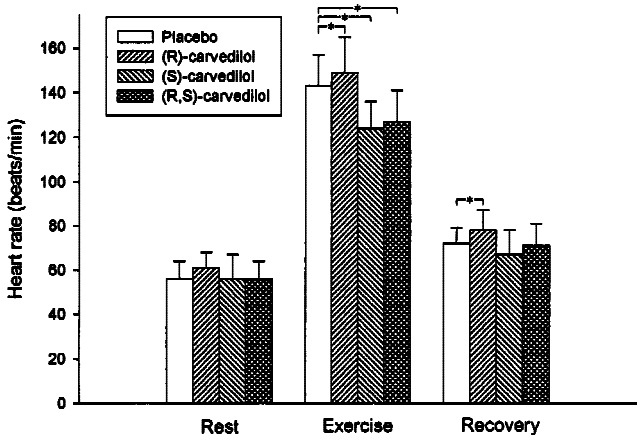

Fig. 1. Heart rate obtained at rest, after 10 min of exercise, and after 15 of recovery, starting 3 h after oral administration of placebo, 12.5 mg (R)-carvedilol, 12.5 mg (S)-carvedilol and 25 mg (R,S)-carvedilol, respectively. Bars = SD; *$P < 0.05$.

On the other hand, heart rate was slightly increased by (R)-carvedilol during exercise as well as after 15 min of recovery. This rise in heart rate most likely may be caused by an increase of sympathetic tone as a physiological reaction to vasodilation resulting from the alpha-blocking effect of (R)-carvedilol. Since both (R)- and (S)-carvedilol have been shown to be effective antagonists of adrenergic alpha-receptors,[5,6] this reflex increase of sympathetic tone might be one reason why both (S)- and (R,S)-carvedilol failed to cause a significant decrease of heart rate at rest and during recovery in the present study. However, there may be several competing factors that might account for the lack of effect of (S)- and (R,S)-carvedilol on heart rate at rest, including the lack of inverse antagonist activity shown with (R,S)-carvedilol[18,19] and the low sympathetic tone at rest in the present study performed in healthy subjects. Furthermore, intrinsic sympathomimetic activity (ISA) might hinder a decreasing effect of (S)- and (R,S)-carvedilol on heart rate at rest. However, (R,S)-carvedilol was shown to lack ISA.[4,18] In addition, it has to be emphasized that our data were obtained in healthy volunteers following oral administration of single doses of the drugs, and greater beta-blocking effects might possible be expected when long-term treatment with the drugs is performed in patients with a higher sympathetic tone at rest. On the other hand, both (R,S)-carvedilol and half-dosed optically pure (S)-carvedilol decreased heart rate during exercise to approximately the same extent. Perhaps the increase of sympathetic tone caused by vasodilation result-

ing from the alpha-blocking effects of both (R)- and (S)-carvedilol is high enough to counteract the blockade of beta-adrenoceptors in healthy subjects at rest. However, during the pronounced stimulation of beta-adrenoceptors during exercise this slight increase of sympathetic tone is no longer detectable when (S)- or (R,S)-carvedilol are given.

Our finding that racemic (R,S)-carvedilol decreased heart rate only during exercise but not at rest is in contrast to data obtained after long-term therapy with (R,S)-carvedilol in patients with myocardial infarction[20] or congestive heart failure,[16] where (R,S)-carvedilol significantly decreased overall heart rate. In addition, a recent trial in patients suffering from dilated cardiomyopathy showed that resting heart rate in patients on long-term treatment with 74 ± 6 mg (R,S)-carvedilol was as low as with 142 ± 11 mg (R,S)-metoprolol, a beta-blocker without intrinsic sympathomimetic activity and without additional alpha-blocking effects.[21] On the other hand, intravenous single-dose administration of (R,S)-carvedilol in healthy subjects[2] led to a pronounced transient increase in heart rate, whereas (R,S)-carvedilol markedly suppressed isoproterenol-induced increase in heart rate. These findings are in good accordance with our present results showing a smaller increase in heart rate during exercise with (R,S)- and (S)-carvedilol compared to (R)-carvedilol and placebo. The finding that (R,S)-carvedilol decreased heart rate at rest only in patients with myocardial infarction or congestive heart failure after long-term treatment but not in healthy subjects after single-dose administration might be explained by the fact that patients with myocardial infarction or congestive heart failure usually have a higher sympathetic tone at rest than healthy volunteers, thus being permanently exposed to elevated sympathetic tone. Furthermore, metabolites with beta-blocking effects might account for the higher beta-blocking potency of (R,S)-carvedilol during long-term treatment compared to single-dose administration.[4]

Neither optically pure (R)- nor (S)-carvedilol nor racemic (R,S)-carvedilol had any significant influence on diurnal or nocturnal melatonin release in our study. Melatonin, the main product of the pineal gland, is synthesized and released mainly at night. This process is stimulated predominantly via adrenergic $beta_1$-receptors, with some additional effects of adrenergic alpha-receptors.[22] Therefore, we determined daytime melatonin release in order to investigate a possible direct stimulating effect of one of the carvedilol enantiomers. However, our results exclude this possibility since we observed no change of melatonin release compared to placebo. On the other hand, nocturnal melatonin release also remained unaffected by (S)- and (R,S)-carvedilol, suggesting a weak net clinical beta-blocking effect at night, i.e., at rest, in healthy subjects. In contrast, (R)-carvedilol showed a slight but nonsignificant tendency to increase nocturnal melatonin release (+24%, n.s.): as with the increase of heart rate during recovery, this finding might best be explained by a slight increase of sympathetic tone resulting from vasodilation caused by the alpha-blocking effects of (R)-carvedilol and causing an increased stimulation of melatonin release.

A further explanation of these effects might be an intrinsic sympathomimetic activity (ISA) of (R)- and (S)-carvedilol. However, it has been repeatedly shown that racemic (R,S)-carvedilol lacks ISA.[4,18,23]

In summary, our findings help to explain the lack of any significant effect of currently used (R,S)-carvedilol on melatonin release even though both production and release of melatonin are stimulated via $beta_1$-adrenoceptors,[22] since a substance which does not exert enough net clinical beta-blockade to reduce heart rate at rest cannot be expected to sufficiently suppress beta-adrenoceptor-mediated melatonin release. This finding is in accordance with the fact that neither (S)- nor (R,S)-carvedilol decrease nocturnal melatonin release in contrast to the pronounced decreases caused by (S)-propranolol and (S)-atenolol (–80% and –86%, respectively) found in a previous study by our group using a similar protocol,[8] in which (R,S)-carvedilol was also found not to affect nocturnal melatonin release.

In conclusion, our results indicate that only (S)-carvedilol causes significant beta-blockade in humans when clinical doses of the drug are used, whereas (R)-carvedilol appears to increase sympathetic tone. Our data further show that the currently used racemic mixture, (R,S)-carvedilol, exerts clinically detectable beta-blockade in healthy subjects only during exercise, whereas it does not significantly reduce heart rate at rest. These findings may explain why neither (S)- nor (R,S)-carvedilol—in contrast to other beta-blockers—reduce nocturnal melatonin release, and why (R,S)-carvedilol may exert fewer side effects resulting from beta-blockade than other ('pure') beta-blockers.

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