in general agreement with the results of monitoring heart rate from other groups,[7,8] although direct comparisons are difficult because of the different methods used and our emphasis on the importance of sustained periods of appropriate physical activity. Our results, in accord with previous findings from The Netherlands,[9,10] suggest that girls are less physically active than similarly aged boys and that girls' level of physical activity decreases while they are at secondary school.

No significant relation was detected between the level of habitual physical activity (heart rate) and skinfold thickness in either sex. Similarly, the children classified as overweight according to the criteria of the Royal College of Physicians[11] were not significantly less active than children who were not overweight.

In conclusion, we have shown that British children have surprisingly low levels of habitual physical activity and that many children seldom experience the intensity and duration of physical activity that are believed to stress the cardiopulmonary system appropriately. The pubertal stage of development or measures of body fatness, or both, do not seem to be sensitive indicators of volume of physical activity in either girls or boys. More research into the determinants of children's patterns of physical activity is required, and, in the light of our data, the effects of short periods (five minutes) of physical activity on children's cardiopulmonary systems need further investigation.

We acknowledge the technical help of Jenny Frost, Alison Husband, Pat Bond, and Clive Williams and the logistic support of Mark Palmer and Maralyn Kempson. The indexing of maturity was carried out by Dr Sarah Hannington. The work was supported by the Northcott Devon Medical Foundation and the IBM (UK) Trust.

1 Simons-Morton BG, Parcel GS, O'Hara NM, Blair SN, Pate RR. Health-related physical fitness in childhood. *Annu Rev Public Health* 1988;9: 403-25.
2 Office of Population Censuses and Surveys. *Census. Small area statistics.* London: HMSO, 1981.
3 Weiner JS, Lourie JA, eds. *Practical human biology.* London: Academic Press, 1981.
4 Tanner JM. *Growth at adolescence.* 2nd ed. Oxford: Blackwell Scientific, 1962.
5 Dickenson B. The physical activity patterns of young people—the implications for PE. *Bulletin of Physical Education* 1986;22:36-9.
6 Williams A. Physical activity patterns among adolescents—some curriculum implications. *Physical Education Review* 1988;11:28-39.
7 Seliger VS, Trefny S, Bartenkova S, Pauer M. The habitual physical activity and fitness of 12 year old boys. *Acta Paediatrica Belgica* 1974;28:54-9.
8 Atomi Y, Iwaoka K, Hatta H, Miyashita M, Yamamoto Y. Daily physical acitivity levels in preadolescent boys related to $Vo_2$ max and lactate threshold. *Eur J Appl Physiol* 1986;55:156-61.
9 Saris WHM. *Aerobic power and daily physical activity in children.* Meppel, The Netherlands: Kripps Repro, 1982.
10 Verschuur R, Kemper HCG. Habitual physical activity. *Medicine and Sport Science* 1985;20:56-65.
11 Royal College of Physicians. Obesity. *J R Coll Physicians Lond* 1983;17:3-58.

*(Accepted 23 May 1990)*

# Prolonged blood pressure reduction by orally active renin inhibitor RO 42-5892 in essential hypertension

A H van den Meiracker, P J J Admiraal, A J Man in 't Veld, F H M Derkx, H J Ritsema van Eck, P Mulder, P van Brummelen, M A D H Schalekamp

**Department of Internal Medicine I, University Hospital Dijkzigt, 3015 GD Rotterdam, The Netherlands**
A H van den Meiracker, MD, *consultant in internal medicine*
P J J Admiraal, MSC, *research fellow*
A J Man in 't Veld, MD, *professor of cardiovascular pharmacology*
F H M Derckx, MD, *consultant in clinical pharmacology*
H J Ritsema van Eck, MD, *consultant in cardiology*
M A D H Schalekamp, MD, *professor of internal medicine*

**Department of Biostatistics, University Hospital Dijkzigt, 3015 GD Rotterdam, The Netherlands**
P Mulder, MSC, *statistician*

**F Hoffmann-La Roche and Co Ltd, Basle, Switzerland**
P van Brummelen, MD, *professor of pharmacology*

Correspondence to:
Professor M A D H Schalekamp, Department of Internal Medicine I, University Hospital Dijkzigt, Room H362, Dr Molewaterplein 40, 3015 GD Rotterdam, The Netherlands.

*Br Med J* 1990;301:205-10

## Abstract

***Objective*—To investigate the effects of a novel specific renin inhibitor, RO 42-5892, with high affinity for human renin ($K_i = 0{\cdot}5 \times 10^{-9}$ mol/l), on plasma renin activity and angiotensin II concentration and on 24 hour ambulatory blood pressure in essential hypertension.**

***Design*—Exploratory study in which active treatment was preceded by placebo.**

***Setting*—Inpatient unit of teaching hospital.**

***Patients*—Nine men with uncomplicated essential hypertension who had a normal sodium intake.**

***Interventions*—Two single intravenous doses of RO 42-5892 (100 and 1000 μg/kg in 10 minutes) given to six patients and one single oral dose (600 mg) given to the three others as well as to three of the patients who also received the two intravenous doses.**

***Results*—With both intravenous and oral doses renin activity fell in 10 minutes to undetectably low values, while angiotensin II concentration fell overall by 80-90% with intravenous dosing and by 30-40% after the oral dose. Angiotensin II concentration was back to baseline four hours after the low and six hours after the high intravenous dose and remained low for at least eight hours after the oral dose. Blood pressure fell rapidly both after low and high intravenous doses and after the oral dose and remained low for hours. With the high intravenous dose the daytime (0900-2230), night time (2300-0600), and next morning (0630-0830) systolic blood pressures were significantly ($p<0{\cdot}05$) lowered by 12·5 (95% confidence interval 5·6 to 19·7), 12·2 (5·4 to 19·3), and 10·7 (3·2 to 18·5) mm Hg respectively, and daytime diastolic pressure was lowered by 9·3 (2·2 to 16·8) mm Hg. With the oral dose daytime, night time, and next morning systolic blood pressures were lowered by 10·3 (5·5 to 15·4), 10·5 (4·2 to 17·2), and 9·7 (4·0 to 15·6) mm Hg, and daytime and night time diastolic pressures were lowered by 5·8 (0·9 to 11·0) and 6·0 (0·3-12) mm Hg respectively.**

***Conclusions*—The effect of the inhibitor on blood pressure was maintained over a longer period than its effect on angiotensin II. RO 42-5892 is orally active and has a prolonged antihypertensive effect in patients who did not have sodium depletion. This prolonged effect seems to be independent, at least in part, of the suppression of circulating angiotensin II.**

## Introduction

The efficacy of drugs that interfere with the formation of the vasoconstrictor octapeptide angiotensin II is well established. Angiotensin II is formed in two steps. The decapeptide angiotensin I is generated from angiotensinogen by the proteolytic activity of the aspartyl protease renin, and then angiotensin converting enzyme converts angiotensin I to II. The beneficial results obtained with angiotensin converting enzyme inhibitors in the management of hypertension and heart failure have stimulated the search for inhibitors of renin, which is the rate limiting enzyme for the formation of plasma angiotensin II.

Detailed knowledge of the tertiary structure of renin has led to the recent advent of high affinity inhibitors specific for renin. These inhibitors, when given intravenously to normotensive subjects, effectively reduce plasma renin activity and angiotensin II.[1-5] Two such compounds have been shown to lower blood pressure, but this effect was short lived.[1,2] RO 42-5892 is a novel renin substrate analogue with high affinity for human

renin ($K_i = 0{\cdot}5 \times 10^{-9}$ mmol/l) and little or no affinity for other aspartyl proteases. Limited experience in healthy volunteers indicates that, given orally, this compound is absorbed from the gastrointestinal tract and causes suppression of plasma renin activity and plasma angiotensin II (unpublished data). In an exploratory study we investigated the effects of two intravenous doses and one single oral dose of this compound on plasma renin activity and angiotensin II as well as on 24 hour ambulatory blood pressure in subjects with essential hypertension.

## Subjects and methods

Nine male patients aged 32 to 55 years with uncomplicated essential hypertension (sitting untreated diastolic blood pressure in the outpatient clinic after 30 minutes' supine rest 100-115 mm Hg, Korotkoff phase V) gave written informed consent to participate in the study, which was approved by the hospital ethical review committee. Antihypertensive drugs were gradually withdrawn over a period of four weeks. The patients maintained their normal diet but were advised not to add extra salt to their food and to avoid salty products. After a drug free period of four weeks 24 hour blood pressure measurements were made while a placebo and the active drug were given. Six of the nine patients received an intravenous saline infusion as placebo, which was followed 24 hours later by an intravenous dose of 100 μg/kg of RO 42-5892. Seven to 14 days later these six patients received a high intravenous dose (1000 μg/kg) of the inhibitor, and three of these six patients were studied for a third time to investigate the effects of a single oral dose of 600 mg. The three remaining patients received only a single oral dose of 600 mg of the inhibitor, and they were studied twice, 7 to 14 days apart, first with the placebo and then with the active drug. The doses we used were based on a preliminary study in normotensive volunteers that showed that these doses significantly reduced plasma renin activity and angiotensin II concentration and had an uncertain effect on blood pressure (unpublished data).

Placebo (0·9% sodium chloride) and RO 42-5892 (total volume 45 ml) were infused over a period of 10 minutes through an indwelling cannula in a forearm vein by means of an infusion pump (Perfusor VI, B Braun Melsungen AG, Melsungen, Germany). For oral dosing RO 42-5892 was dissolved in 150 ml orange juice.

Intra-arterial blood pressure and heart rate were measured for 24 hours. To obtain optimally standardised conditions the patients stayed in hospital during the taking of blood pressure measurements as well as the preceding evening and night. Blood pressure measurements were always started at 0830 after an overnight fast. Placebo or active drug was given at 0850 (zero time). Arterial blood samples for measuring renin activity and angiotensin II concentration were taken at −10, 0, 20, 30, 40, 60, 120, 180, 240, 360, 480, and 1440 minutes. During the first eight hours of the study the patients were lying in bed in the cardiovascular laboratory. Thereafter they were allowed to walk around freely, but they remained in the hospital. Two standard meals were provided at 1300 and 1730. The patients went to sleep at 2330 and woke up at 0600.

For the blood pressure measurements a 10 cm long 1·0 mm diameter Teflon catheter was introduced in the brachial artery of the non-dominant arm after local anaesthesia with 2% lidocaine. The catheter was connected to a miniature transducer-perfusion device fitted in front of the chest. The transducer signal was recorded on magnetic tape by means of a portable tape recorder (Medilog Recorder II, Oxford Medical Instruments, Oxford). The recorded signal was analysed beat by beat as described previously.[6] The average blood pressure and heart rate over 30 minutes were computed for each 24 hour period. In addition, averages over three minutes were calculated for the first 40 minutes after giving placebo or RO 42-5892 to monitor more closely the onset of the antihypertensive effect of the drug.

For measuring plasma renin activity 5 ml blood was collected in tubes containing 0·1 ml of 0·7 M sodium citrate. Plasma renin activity was measured as the rate of angiotensin I formation (nmol/l/h) during incubation of endogenous renin and substrate at neutral pH and 37°C.[7] The angiotensin I formed was measured by radioimmunoassay. The lower limit of detection value of plasma renin activity was 0·05 nmol/l/h.

For measuring plasma angiotensin II concentration 5 ml blood was collected in chilled tubes containing 0·25 ml of an inhibitor solution (6·265 mM disodium EDTA, 0·0001 mM of the renin inhibitor CGP 29, 287, and 1·25 mM 1,10-phenantroline, final concentrations

TABLE I—*Patient characteristics during administration of placebo*

| Case No | Age (years) | Indirect blood pressure (mm Hg) | 24 Hour urinary sodium excretion (mmol/day) | Plasma renin activity (nmol/l/h) | Plasma angiotensin II concentration (mol/l) |
|---|---|---|---|---|---|
| 1 | 32 | 147/103 | 92 | 0·41 | 2·4 |
| 2 | 35 | 175/103 | 66 | 1·28 | 8·5 |
| 3 | 39 | 147/108 | 58 | 0·41 | 2·0 |
| 4 | 48 | 160/108 | 128 | 3·06 | 12·0 |
| 5 | 49 | 175/103 | 81 | 2·01 | 4·4 |
| 6 | 49 | 154/102 | 100 | 1·18 | 5·9 |
| 7 | 55 | 161/101 | 81 | 0·64 | 1·4 |
| 8 | 48 | 180/114 | 129 | 1·05 | 4·1 |
| 9 | 55 | 180/105 | 50 | 0·35 | 1·9 |

TABLE II—*Geometric mean (range) of plasma renin activity and plasma angiotensin II concentration during administration of placebo and after administration of intravenous RO 42 5892 in six subjects with essential hypertension*

| | Placebo | | Intravenous RO 42-5892 | | | |
|---|---|---|---|---|---|---|
| | | | 100 μg/kg | | 1000 μg/kg | |
| Time (min) | Plasma renin activity (nmol/l/h) | Angiotensin II concentration (pmol/l) | Plasma renin activity (nmol/l/h) | Angiotensin II concentration (pmol/l) | Plasma renin activity (nmol/l/h) | Angiotensin II concentration (pmol/l) |
| −10 | 0·94 (0·41-3·17) | 6·2 (1·9-15·3) | 1·24 (0·52-2·63) | 5·6 (2·2-12·9) | 0·63 (0·41-2·08) | 4·0 (1·6- 8·3) |
| 0 | 0·94 (0·35-3·55) | 5·3 (2·0-13·6) | 1·18 (0·53-2·71) | 6·7 (2·7-14·1) | 0·69 (0·53-2·11) | 3·7 (1·6- 9·1) |
| 10 | 0·92 (0·33-3·30) | 5·7 (1·8-12·8) | 0·05 (0·05-0·05) | 0·4 (0·3- 0·8) | 0·05 (0·05-0·05) | 0·4 (0·3- 0·5) |
| 20 | 0·88 (0·40-3·14) | 5·3 (1·2-10·7) | 0·05 (0·05-0·05) | 1·1 (0·3- 4·4) | 0·05 (0·05-0·07) | 0·4 (0·3- 0·7) |
| 30 | 0·95 (0·37-3·02) | 5·0 (1·4-12·3) | 0·08 (0·05-0·19) | 1·7 (0·5- 4·4) | 0·06 (0·05-0·08) | 0·4 (0·3- 1·0) |
| 40 | 0·87 (0·38-2·99) | 4·7 (1·9- 9·3) | 0·12 (0·05-0·50) | 1·8 (0·4- 6·2) | 0·05 (0·05-0·13) | 0·5 (0·3- 0·9) |
| 60 | 0·89 (0·30-3·23) | 5·2 (2·1-11·3) | 0·15 (0·08-0·33) | 3·2 (0·8- 7·8) | 0·05 (0·05-0·05) | 0·9 (0·4- 1·3) |
| 120 | 0·85 (0·34-2·63) | 5·4 (1·3-15·6) | 0·20 (0·05-0·66) | 4·1 (1·2- 8·0) | 0·07 (0·05-0·08) | 1·3 (0·6- 3·7) |
| 180 | 0·95 (0·36-2·31) | 6·5 (1·6-15·2) | 0·30 (0·10-0·78) | 4·7 (1·8- 8·5) | 0·08 (0·05-0·09) | 1·6 (1·0- 3·4) |
| 240 | 0·90 (0·50-3·73) | 6·9 (2·1-11·8) | 0·41 (0·12-1·28) | 5·4 (2·0- 9·9) | 0·07 (0·05-0·13) | 2·9 (1·8- 3·9) |
| 360 | 1·16 (0·32-2·75) | 7·2 (2·3-14·2) | 0·80 (0·35-2·75) | 7·5 (3·4-11·4) | 0·18 (0·13-0·29) | 4·0 (2·7- 6·4) |
| 480 | 0·87 (0·53-2·71) | 4·6 (1·4-15·0) | 0·71 (0·33-2·06) | 6·2 (2·9-10·6) | 0·15 (0·09-0·56) | 2·3 (0·5- 5·9) |
| 1440 | 1·18 (0·53-2·71) | 5·8 (2·2-12·9) | 1·24 (0·52-2·19) | 7·5 (3·6-14·5) | 0·49 (0·31-1·15) | 5·1 (2·3-10·2) |

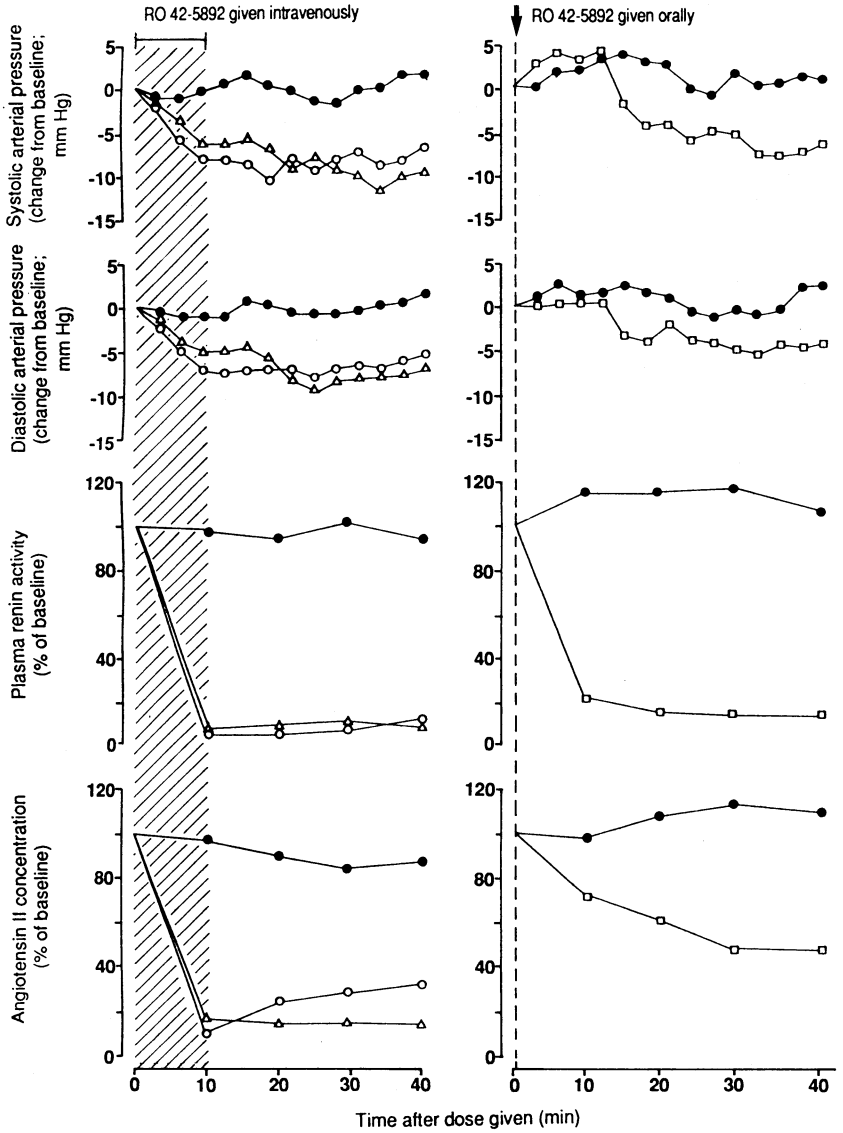


FIG 1—*Initial effects of RO 42-5892 on systolic and diastolic blood pressure, plasma renin activity, and angiotensin II concentration. Doses were: 100 μg/kg intravenously, (○), 1000 μg/kg intravenously (△), and 600 mg orally (□) in six subjects with essential hypertension. Shaded area indicates the infusion period and dotted line indicates the time of oral administration. (●)=Placebo*

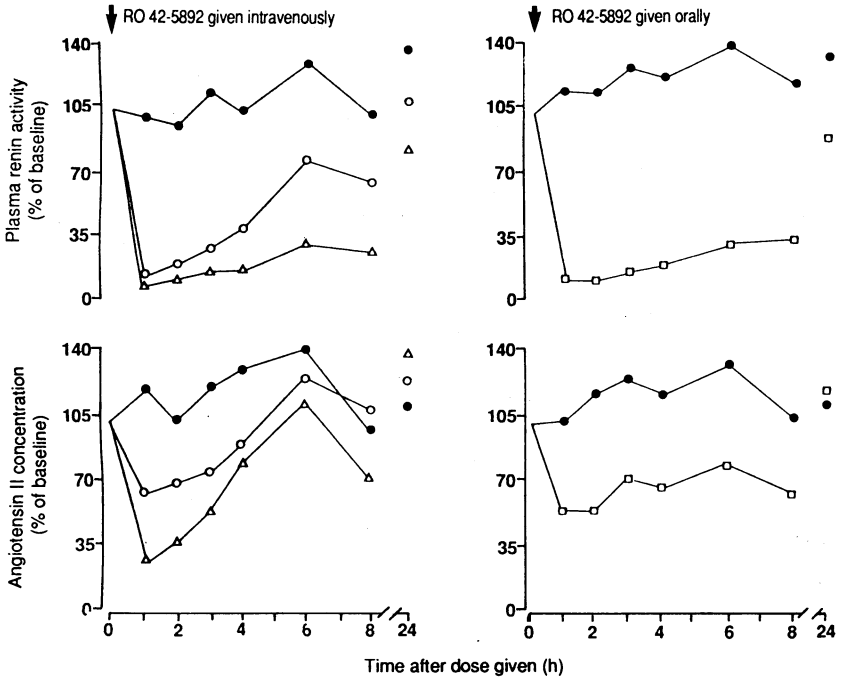


FIG 2—*Percentage changes in plasma renin activity and angiotensin II concentration after RO 42-5892 100 μg/kg intravenously (○), 1000 μg/kg intravenously (△), and 600 mg orally (□) was given to six subjects with essential hypertension. Arrows indicate the time of dose. (●)=Placebo*

in blood).[8] The blood samples were immediately centrifuged at 5000 *g* for 10 min at 4°C. Plasma was stored at −70°C. Within two days after sampling angiotensins were extracted from 2 ml plasma with Sep Pak C18 cartridges (Waters, Millford, Massachusetts, United States). Separation of true angiotensin II from cross reacting metabolites was performed by high performance liquid chromatography according to the method of Nussberger *et al*[9] with some modifications.[8] The lower limit of detection value of angiotensin II was 0·3 pmol/l.

At the end of each study blood samples were taken for routine haematological and biochemical measurements: haemoglobin concentration; packed cell volume; red and white blood cell and platelet counts; serum concentrations of creatinine, urea nitrogen, electrolytes, and total bilirubin and activities of alanine aminotransferase, aspartate aminotransferase, γ-glucosyltransferase, and alkaline phosphatase; and blood glucose concentration.

For statistical analysis the 24 hour ambulatory blood pressure recordings were grouped into three time periods: 0900-2230 (daytime), 2300-0600 (night time); and 0630-0830 (next morning). When the plasma renin activity or angiotensin II concentration, or both, fell below the lower limit of detection of our laboratory, after administration of the renin inhibitor, the value of the unit detected was used for calculating mean values. For plasma renin activity and angiotensin II concentration the geometric means and extreme values are given, as values were not distributed normally. The changes in plasma renin activity and angiotensin II concentration that occurred after renin inhibition were compared with average baseline values. Student's two tailed paired *t* test was used for comparison. p Values of $<0·05$ were considered significant.

## Results

Table I gives plasma renin activity, angiotensin II concentration, 24 hour urinary sodium excretion on the day of giving placebo, and pretreatment values or indirectly measured blood pressure. The renin inhibitor was well tolerated, and adverse effects of haematological or biochemical abnormalities were not observed.

Immediately after intravenous doses of RO 42-5892 the plasma renin activity fell to undetectably low values and angiotensin II concentration fell by 80-90% (fig 1, table II). The suppressive effect of RO 42-5892 on plasma renin activity and angiotensin II concentration took longer to wear off with the high than with the low intravenous dose (fig 2, table II). With both intravenous doses the plasma renin activity remained low compared with average baseline values ($p<0·01$) for at least eight hours, but 24 hours after dosing it was back to baseline. Plasma angiotensin II concentration was no longer significantly different from baseline four hours after the low and six hours after the high intravenous dose. Plasma renin activity and angiotensin II concentration fell rapidly, within 10 minutes, after the oral dose (fig 1, table III). Both plasma renin activity and angiotensin II concentration remained low ($p<0·05$) for at least eight hours and were back to baseline values after 24 hours. With intravenous as well as with oral doses plasma renin activity remained suppressed for a longer time than angiotensin II concentration.

Baseline blood pressures before giving placebo and RO 42-5892 intravenously or orally were not different. With both low and high intravenous doses of RO 42-5892 blood pressure began to fall during the 10 minute infusion period whereas with oral RO 42-5892 it began to fall 10 to 20 minutes after administration (fig 1). Figure 3 shows the 24 hour plots of blood pressure and heart rate after placebo and after the two intravenous

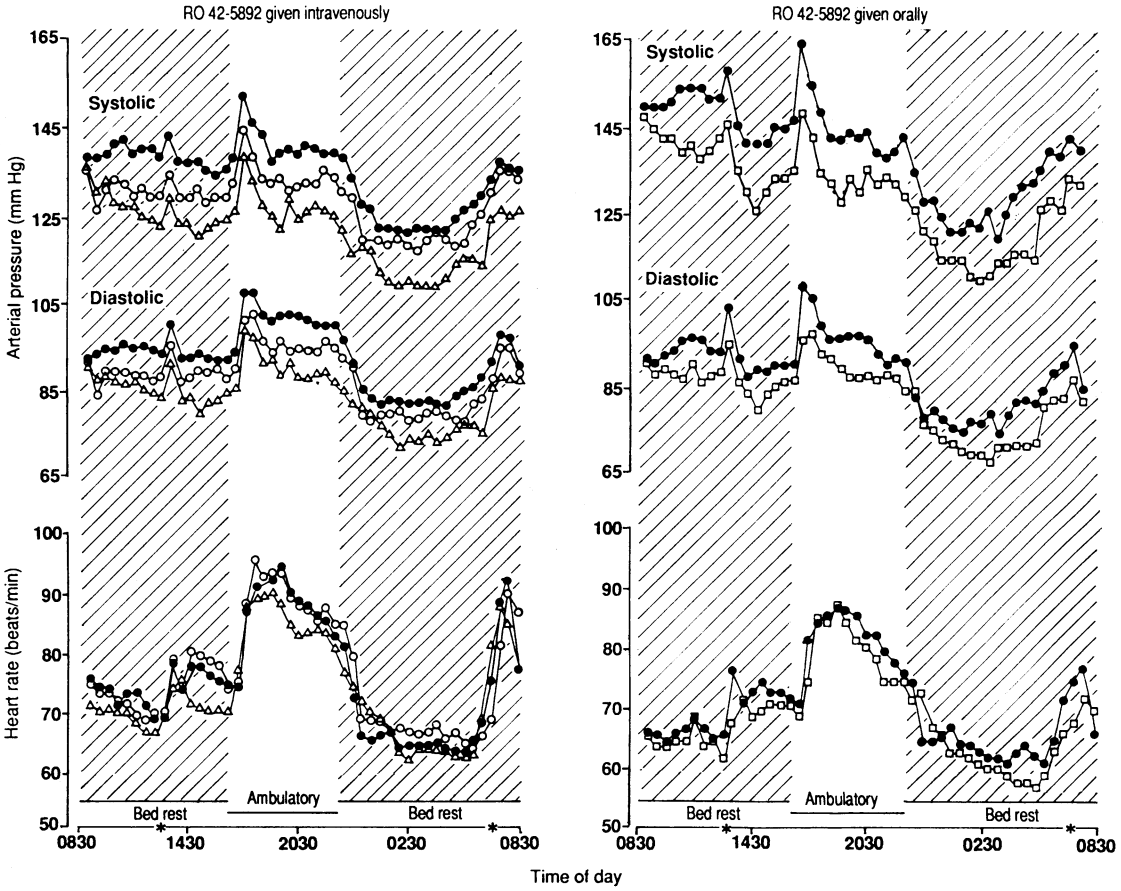


FIG 3—*Effects of RO 42-5892 on 24 hour blood pressure and heart rate profiles in six subjects with essential hypertension. (●)=Placebo; (○)=RO 42-5892 100 μg/kg intravenously; (△)=RO 42-5892 1000 μg/kg intravenously; (□)=RO 42-5892 600 mg orally; times at which patients had lunch and were awakened are indicated by asterisks*

doses and the oral dose of RO 42-5892. Both the high intravenous dose and oral dose caused a longlasting fall in blood pressure. Individual and mean responses of daytime, night time, and next morning blood pressures to RO 42-5892 are summarised in table IV. The blood pressure responses to RO 42-5892 were not accompanied by changes in heart rate.

## Discussion

This study shows that the renin inhibitor RO 42-5892, when given intravenously, effectively suppresses the formation of plasma angiotensin II in subjects with essential hypertension. The compound was also effective when given orally. Not only after intravenous doses but also after oral dosing the effects on plasma renin activity and angiotensin II concentration were evident after ten minutes. Thus the inhibitor is rapidly absorbed.

TABLE III—*Geometric mean (range) of plasma renin activity and plasma angiotensin II concentration during administration of placebo and after oral administration of 600 mg RO 42-5892 in six subjects with essential hypertension*

| Time (min) | Placebo | | Oral RO 42-5892 | |
|---|---|---|---|---|
| | Plasma renin activity (nmol/l/h) | Angiotensin II concentration (pmol/l) | Plasma renin activity (nmol/l/h) | Angiotensin II concentration (pmol/l) |
| −10 | 0·83 (0·33-3·17) | 4·3 (1·7-12·3) | 0·71 (0·38-1·18) | 3·6 (1·8-10·0) |
| 0 | 0·80 (0·19-3·55) | 3·8 (1·0-13·6) | 0·68 (0·36-1·14) | 3·9 (1·8- 9·1) |
| 10 | 0·94 (0·35-3·30) | 3·8 (1·5-12·8) | 0·11 (0·05-0·32) | 2·5 (1·0- 8·7) |
| 20 | 0·92 (0·45-3·30) | 3·6 (1·3-10·7) | 0·08 (0·05-0·29) | 1·8 (0·4- 5·2) |
| 30 | 0·95 (0·34-3·14) | 3·7 (1·6-12·3) | 0·09 (0·05-0·16) | 1·4 (0·4- 2·2) |
| 40 | 0·86 (0·36-3·02) | 3·6 (1·5- 9·3) | 0·08 (0·05-0·12) | 1·5 (0·5- 2·7) |
| 60 | 0·91 (0·34-2·99) | 3·8 (1·2-11·3) | 0·06 (0·05-0·12) | 1·6 (0·7- 3·9) |
| 120 | 0·89 (0·34-3·23) | 4·6 (1·5-15·6) | 0·07 (0·05-0·17) | 1·4 (0·5- 5·6) |
| 180 | 1·00 (0·39-3·36) | 4·5 (1·0-15·2) | 0·09 (0·05-0·25) | 2·6 (1·6- 5·2) |
| 240 | 0·93 (0·32-2·31) | 4·1 (1·3-11·8) | 0·13 (0·07-0·25) | 2·4 (1·2- 5·6) |
| 360 | 1·15 (0·51-3·73) | 4·8 (1·2-14·2) | 0·19 (0·14-0·35) | 2·9 (1·4- 5·5) |
| 480 | 0·95 (0·32-2·75) | 3·7 (1·5-15·0) | 0·20 (0·15-0·34) | 2·4 (1·3- 5·3) |
| 1440 | 1·02 (0·32-2·71) | 4·4 (1·8- 8·6) | 0·56 (0·39-0·96) | 3·8 (1·9- 6·7) |

The changes in plasma renin activity and angiotensin II concentration in response to RO 42-5892 did not run in parallel. After a rapid initial fall both plasma renin activity and angiotensin II concentration returned gradually to baseline values, but it took longer for plasma renin activity than angiotensin II concentration to return. This has also been noted with another renin inhibitor, CGP 38560 A.[3,5] The in vitro measurement of plasma renin activity of subjects treated with renin inhibitor may not be a reliable index of the in vivo plasma renin activity; the assay of plasma renin activity may overestimate the in vivo inhibition of activity.[5] An alternative explanation could be that angiotensin II is formed by enzymes other than renin.[10-12]

RO 42-5892 caused long lasting reduction in blood pressure after both intravenous and oral dosing. If the antihypertensive action of the inhibitor depends solely on its effect on plasma angiotensin II then a strict parallel between the two would be expected. It is true that the immediate fall in angiotensin II concentration coincided with a fall in blood pressure, but blood pressure was still reduced at the time angiotensin II concentration had returned to baseline values. This was seen with both intravenous and oral doses. It seems likely, therefore, that the effect of the inhibitor on blood pressure is not mediated only by its action on the circulating renin-angiotensin system. Evidence is accumulating for the existence of a vascular renin-angiotensin system, which coexists with circulating renin and angiotensin.[13-15] Experimental studies indicate that this vascular system participates in the local control of vascular tone.[16] Saito *et al* recently showed that the local generation of angiotensin II in isolated perfused rat mensenteric arteries is suppressed during infusion of *N*-acetyl-pepstatin, an inhibitor of renin.[17] It is conceivable that the prolonged antihypertensive effect of RO 42-5892, as observed in our study, is caused by inhibition of the vascular renin-

TABLE IV — *Individual daytime, night time, and next morning systolic (SAP) and diastolic (DAP) blood pressures during placebo and responses to intravenous or oral RO 42-5892 in subjects with essential hypertension*

| | Placebo | | Change in blood pressure after RO 42-5892 | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 100 μg/kg intravenously | | 1000 μg/kg intravenously | | 600 mg orally | |
| Case No | SAP (mm Hg) | DAP (mm Hg) | SAP (mm Hg) | DAP (mm Hg) | SAP (mm Hg) | DAP (mm Hg) | SAP (mm Hg) | DAP (mm Hg) |
| | | | | | *Day time (0900-2230)* | | | |
| 1 | 124 | 93 | −5 | −3 | −6 | 2 | | |
| 2 | 134 | 94 | −3 | −3 | −9 | −9 | | |
| 3 | 142 | 100 | −9 | −9 | −12 | −12 | | |
| 4 | 162 | 107 | −11 | −6 | −15 | −10 | −7 | −4 |
| 5 | 135 | 91 | −11 | −8 | −8 | −7 | −8 | −7 |
| 6 | 140 | 98 | −6 | −5 | −25 | −20 | −20 | −15 |
| 7 | 140 | 88 | | | | | −8 | −3 |
| 8 | 155 | 100 | | | | | −10 | −5 |
| 9 | 149 | 88 | | | | | −9 | −1 |
| Mean (95% confidence interval) | | | −7·5** (−4·2 to −11·0) | −5·7** (−3·2 to −8·3) | −12·5** (−5·6 to −19·7) | −9·3* (−2·2 to −16·8) | −10·3** (−5·5 to −15·4) | −5·8* (−0·9 to −11·0) |
| | | | | | *Night time (2300-0600)* | | | |
| 1 | 119 | 88 | 2 | 2 | −1 | 7 | | |
| 2 | 125 | 83 | 2 | 1 | −14 | −9 | | |
| 3 | 123 | 84 | −14 | −12 | −9 | −8 | | |
| 4 | 145 | 92 | −8 | −5 | −16 | −9 | −8 | −4 |
| 5 | 116 | 76 | −9 | −5 | −12 | −9 | −13 | −8 |
| 6 | 122 | 82 | −1 | −2 | −21 | −17 | −15 | −11 |
| 7 | 107 | 64 | | | | | 0 | 2 |
| 8 | 139 | 91 | | | | | −18 | −13 |
| 9 | 134 | 77 | | | | | −9 | −2 |
| Mean (95% confidence interval) | | | −4·7 (2·0 to −11·6) | −3·5 (1·6 to −8·8) | −12·2* (−5·4 to −19·3) | −7·5 (0·3 to −15·7) | −10·5** (−4·2 to −17·2) | −6·0* (−0·3 to −12·0) |
| | | | | | *Next morning (0630-0830)* | | | |
| 1 | 122 | 93 | −1 | 0 | −2 | 7 | | |
| 2 | 140 | 96 | −1 | −2 | −15 | −14 | | |
| 3 | 134 | 97 | −4 | −4 | −5 | −3 | | |
| 4 | 150 | 97 | −8 | −7 | −9 | −5 | −8 | −4 |
| 5 | 127 | 87 | 3 | −1 | −10 | −8 | −10 | −8 |
| 6 | 139 | 88 | −4 | −5 | −23 | −19 | −19 | −15 |
| 7 | 135 | 83 | | | | | −2 | 2 |
| 8 | 147 | 97 | | | | | −12 | −10 |
| 9 | 142 | 87 | | | | | −7 | −1 |
| Mean (95% confidence interval) | | | −2·5 (1·2 to −6·4) | −3·2 (−0·5 to −5·9) | −10·7** (−3·2 to −18·5) | −7·0 (2·1 to −16·5) | −9·7** (−4·0 to −15·6) | −6·0 (0·2 to −12·5) |

*p<0·05; **p<0·01 for RO 42-5892 *v* placebo.

angiotensin system rather than by its action on the circulating system.

The antihypertensive effect of RO 42-5892 was not accompanied by reflex tachycardia, a finding also reported in animal studies with renin inhibitors.[18-21] At variance with this finding is a study of Webb *et al* in normotensive subjects with the renin inhibitor H142.[1] This inhibitor, given intravenously, lowered blood pressure and raised heart rate. Both effects were short lived. Their subjects, who were pretreated with a single dose of frusemide 40 mg orally followed by three days sodium restriction (12-15 mmol/day), were in a sodium depleted state and this may account for the reflex tachycardia.

Our study had an exploratory character and was carried out in a non-randomised way. As placebo was always given before active treatment the observed effect on blood pressure may have been related to an order effect. Such an order effect cannot be excluded with absolute certainty, but the following observations indicate that it is probably not very important. Firstly, baseline blood pressures before the placebo and the active drug were given were not different. Secondly, the antihypertensive response to intravenous doses was related to the dose. After the low intravenous dose the antihypertensive effect was evident during the first 12 hours and not during the second half of the day whereas after the high intravenous dose the effect, on systolic arterial pressure at least, was evident during the whole 24 hour period. It should also be noted that a study of the day to day reproducibility of 24 hour intra-arterial ambulatory blood pressure recordings did not find any difference between recordings on the first and second days.[22]

RO 42-5892 belongs to the rapidly expanding class of specific renin inhibitors. It is the first of these compounds shown to have a prolonged effect on blood pressure in hypertensive patients without sodium depletion and to be active when given orally. By virtue of their unique property to inhibit the first, rate limiting, step of angiotensin II formation the renin inhibitors may contribute to our understanding of the role of the renin-angiotensin system in cardiovascular homoeostasis. As suggested by our study, these compounds may also prove useful in treating hypertension and perhaps also heart failure. The high specificity of the newly developed renin inhibitors, as compared to other drugs suppressing angiotensin II formation, may add to their clinical usefulness.

RO 42-5892 was provided by F Hoffmann-La Roche and Co Ltd.

1 Webb DJ, Manhem PJO, Ball SG, *et al*. A study of the renin inhibitor H142 in man. *J Hypertens* 1985;3:653-8.
2 Zusman RM, Burton J, Christensen D, Dodds A, Haber E. Hemodynamic effects of a competitive renin inhibitory peptide in man: evidence for multiple mechanisms of action. *Trans Am Assoc Physicians* 1983;**96**:365-74.
3 De Gasparo M, Cumin F, Nussberger J, Guyenne TT, Wood JM, Menard J. Pharmacological investigations of a new renin inhibitor in normal sodium-unrestricted volunteers. *Br J Clin Pharmacol* 1989;**27**:587-96.
4 Delabays A, Nussberger J, Porchet M, *et al*. Hemodynamic and humoral effects of the new renin inhibitor enalkiren in normal humans. *Hypertension* 1989;**13**:941-7.
5 Nussberger J, Delabays A, De Gasparo M, *et al*. Hemodynamic and biochemical consequences of renin inhibition by infusion of CGP 38560A in normal volunteers. *Hypertension* 1989;**13**:948-53.
6 Van den Meiracker AH, Mann in 't Veld AJ, Ritsema van Eck HJ, Boomsma F, Schalekamp MADH. Hemodynamic and hormonal adaptations to β-adrenoceptor blockade. A 24-hour study of acebutolol, atenolol, pindolol, and propranolol in hypertensive patients. *Circulation* 1988;**78**:957-68.
7 Derkx FHM, Tan-Tjiong HL, Wenting GJ, Boomsma F, Schalekamp MADH. Asynchronuous changes in prorenin and renin secretion after captopril in patients with renal artery stenosis. *Hypertension* 1983;5:244-56.
8 Admiraal PJJ, Derkx FHM, Danser AHJ, Pieterman H, Schalekamp MADH. Metabolism and production of angiotensin I in different vascular beds in subjects with hypertension. *Hypertension* 1990;**15**:44-55.
9 Nussberger J, Brunner DB, Waeber B, Brunner HR. True versus immunoreactive angiotensin II in human plasma. *Hypertension* 1985;**7** (suppl I):1-7.
10 Okunishi H, Miyazaki M, Toda N. Evidence for a putatively new angiotensin II-generating enzyme in the vascular wall. *J Hypertension* 1984;**2**:227-84.
11 Boucher R, Asselin J, Genest J. A new enzyme leading to the direct formation of angiotensin II. *Circ Res* 1974;**34** (suppl 1):203-9.
12 Bund SJ, Aalkjaer C, Heagerty AM, Leckie B, Lever AF. The contractile effects of porcine tetradecapeptide renin substrate in human resistance vessels: evidence of activation by vascular wall renin and serine proteases. *J Hypertens* 1989;7:741-6.
13 Dzau VJ. Significance of the vascular renin-angiotensin pathway. *Hypertension* 1986;**8**:553-9.

14 Campbell DJ. Tissue renin-angiotensin system: sites of angiotensin formation. *J Cardiovasc Pharmacol* 1987;**10** (suppl 7):S1-8.
15 Kifor I, Dzau VJ. Endothelial renin-angiotensin pathway: evidence for intracellular synthesis and secretion of angiotensins. *Circ Res* 1987;**60**: 422-8.
16 Oliver JA, Sciacca RR. Local generation of angiotensin II as a mechanism of regulation of peripheral vascular tone in the rat. *J Clin Invest* 1984;**74**: 1247-51.
17 Saito H, Nakamura M, Ogihar T, *et al.* Renin inhibitor and converting enzyme inhibitors suppress vascular angiotensin II. *Hypertension* 1989;**13**: 749-53.
18 Wood JM, Gulati N, Forgiarini P, Fuhrer W, Hofbauer KG. Effects of a specific and long-acting renin inhibitor in the marmoset. *Hypertension* 1985;7:797-803.
19 Pals DT, Thaisrivongs S, Lawson JA, *et al.* An orally active inhibitor of renin. *Hypertension* 1986;**8**:1105-6.
20 Kleinert HD, Martin D, Chekal MA, *et al.* Cardiovascular actions of the primate-selective renin inhibitor A 62198. *J Pharmacol Exp Ther* 1988;**264**: 975-9.
21 Verburg KM, Kleinert HD, Kadam JRC, Chekal MA, Mento PF, Wilkes BM. Effects of chronic infusion of renin inhibitor A-64662 in sodium depleted monkeys. *Hypertension* 1989;**13**:262-72.
22 Mann S, Millar Craig MW, Balasubramanian V, Cashman PMM, Raftery EB. Ambulant blood pressure: reproducibility and the assessment of interventions. *Clin Sci* 1980;**59**:497-500.

*(Accepted 23 May 1990)*

# Sexual transmission of hepatitis C virus: cohort study (1981-9) among European homosexual men

Mads Melbye, Robert J Biggar, Per Wantzin, Kim Krogsgaard, Peter Ebbesen, Niels G Becker

## Abstract

***Objective*—To determine the prevalence, incidence, and persistence of positivity for antibodies to hepatitis C virus (anti-HCV) and the potential for sexual transmission of the virus.**

***Design*—A cohort analysis covering 1981-9 comparing estimated cumulative incidences of and seroconversion rates for anti-HCV with those of hepatitis B core antibody (anti-HBc) and antibodies to the human immunodeficiency virus (anti-HIV).**

***Setting*—Copenhagen and Aarhus, Denmark.**

***Subjects*—259 Male members of a Danish homosexual organisation.**

***Main outcome measures*—Correlations of prevalence and incidence with a wide range of sexual lifestyle variables.**

***Results*—Only four (1·6%) subjects were positive for anti-HCV in 1981. The estimated cumulative incidence of positivity for anti-HCV was 4·1% in 1984 (seroconversion rate during 1981-4 (2·5%)) and remained at 4·1% in 1989 (seroconversion rate nil during 1984-9). In contrast, positivity for anti-HBC rose from 44·0% in 1981 to 52·7% in 1984 (seroconversion rate 15·5%) and 58·8% in 1989 (serconversion rate 12·9%), and that for anti-HIV rose from 8·8% to 24·0% (seroconversion rate 16·7%) and 30·1% (seroconversion rate 8·0%) respectively. Three anti-HCV positive patients seroreverted three to five years later. None of the anti-HCV positive subjects had had a transfusion and only one gave a past history of intravenous drug use. Variables in sexual lifestyle correlated with the presence of anti-HBc but not with that of anti-HCV.**

***Conclusions*—In contrast with hepatitis B virus and HIV, sexual transmission of hepatitis C virus seems to be a rare event. Furthermore, antibodies to the virus may become undetectable after several years.**

**Department of Infectious Diseases, Rigshospitalet and Institute of Cancer Epidemiology, Danish Cancer Registry, Copenhagen, Denmark**
Mads Melbye, MD, *senior epidemiologist*

**Environmental Epidemiology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, United States**
Robert J Biggar, MD, *professor in epidemiology*

**Blood Bank, Rigshospitalet, Copenhagen, Denmark**
Per Wantzin, MD, *registrar in immunology*

**Department of Rheumatology, Hvidovre Hospital, Copenhagen, Denmark**
Kim Krogsgaard, MD, *registrar in internal medicine*

**Department of Virus and Cancer, Danish Cancer Society, Aarhus, Denmark**
Peter Ebbesen, MD, *chief of department*

**Department of Statistics, La Trobe University, Bundoora, Victoria, Australia**
Niels G Becker, PHD, *professor in statistics*

Correspondence and requests for reprints to: Dr M Melbye, Department of Infectious Diseases, Rigshospitalet, Tagensvej 20, 2200 Copenhagen N, Denmark.

*Br Med J* 1990;**301**:210-2

## Introduction

Although the classification and early descriptions of clinical non-A non-B hepatitis were derived primarily from cases associated with transfusion, this mode of transmission has recently been estimated to account for only 5-10% of patients with the disease in the developed world.[1] In a substantial proportion of cases no obvious route or source of transmission is found. In this respect, therefore, the importance of sexual transmission has been an open question. A possibility for better understanding has emerged with the molecular cloning of an agent designated hepatitis C virus, which seems responsible for most cases of parenteral non-A non-B hepatitis.[2,3] To date frequent transmission of hepatitis C virus by exposure to blood has been described in drug addicts and patients with haemophilia[4-8] whereas little has been reported on the frequency of sexual transmission of the virus. We have studied the prevalence and persistence of antibodies to hepatitis C virus among homosexual men and analysed their possible association with a range of lifestyle factors to see if there is evidence for homosexual spread of hepatitis C virus. Comparison was made with two other viral agents known to be frequently transmitted sexually—namely, hepatitis B virus[9] and HIV.[10]

## Subjects and methods

We studied a cohort of Danish homosexual men who had been followed up since 1981.[10,11] Briefly, 259 subjects were initially enrolled from volunteers belonging to a national organisation of homosexual men. At enrolment their average age was 32·0 years, their average duration of homosexual activity 12·2 years, and their average number of partners 24·9 a year. Cross sectional analyses were performed on all subjects comparing antibody state with lifestyle and demographic variables. In addition, longitudinal studies were done on subjects who subsequent to the 1981 visit were also seen in 1984 or 1989, or in both years. Compared with the original 1981 cohort members of this subset were slightly more experienced sexually (1984, 13·3 years of homosexual activity; 1989, 14·0 years) but otherwise were similar.

A log linear model was used to compute the non-parametric maximum likelihood estimate of the survival curves (1−estimated cumulative antibody incidence) from interval censored data, as detailed elsewhere.[12] We also calculated the seroconversion rates between years of testing using the same methodology. Associations between serological markers and variables in lifestyle were analysed by Spearman rank order correlations and linear regressions.

Serum samples were kept frozen at −70°C until assay and had not previously been thawed. All serum samples were tested for antibodies to hepatitis C virus (anti-HCV) by an enzyme linked immunosorbent assay (ELISA; Ortho Diagnostic Systems). Samples that were non-reactive to hepatitis C virus in the initial run were considered to be anti-HCV negative. Samples that were reactive to hepatitis C virus were retested and considered to be positive if reactive in both tests. Exposure to hepatitis B was determined by an assay for antibodies to hepatitis B core antigen (anti-HBc; CORZYME, Abbott Laboratories). This marker does not recognise patients immunised by vaccination.