バイスが登場しているが、各製品ともに、非常に重要な視点を見逃している。それは医学的なエビデンスと、デバイスの先にあるサービス開発である。医療機関である国立循環器病研究センターが中心に進めることで、ここで得られたデータというのは、全てが開発中デバイスにとって有益なものとなり、かつ、薬事を踏まえた機器開発も可能となる。しかも、我々が開発している機器は薬事承認を目指していることから、現在、上市されているデバイスよりも薬事的な信頼度が非常に高いデバイスとなる。このエビデンスを持った機器を一般の方に使ってもらうような取り組みも合わせて進めており、実際には、医療機関が購入をして外来患者や訪問診療または人間ドックなどで使用することを想定している。さらには、当該モニタリング機器を用いることで、在宅での日常生活上でも簡便に煩わしくなく連続装着が可能であり、日常生活上で血圧を記録することができることから、「施策目標 1-1　日常生活圏の中で良質かつ適切な医療が効率的に提供できる体制を整備すること」と「施策目標 6-1　有効性・安全性の高い新医薬品・医療機器を迅速に提供できるようにすること」への活用の可能性がある。

具体的には、血圧のスクリーニングによって高血圧患者を早期に発見し、治療を開始することで、高血圧から派生する多くの疾患を予防することが可能となる。

我々が開発中のモニタリング機器は、光学的に脈波を検出することから非浸襲で安全であり、更に腕時計として装着するだけなので患者への負担が少なく、且つ診療所や在宅でのモニタリングの活用が期待できると同時に、一般の健常者も気軽に、そして手軽に自身の血圧を測定することで、日々の血圧の上下を意識しながら生活を送り、健康の意識を高めていく期待を持っている。

E．結論

エビデンスが得られることから、我々の開発中デバイスの事業性は非常に高いと言える。また、血圧計の長年の課題でもある「カフ」についても、我々の開発中のデバイスでは、カフ無しを提案していることから、その課題はクリアすることができ、なおかつ、既存のデバイスとの同等の精度を達成することも可能と考えている。

そして、デバイスだけではないサービスの開発について、昨今のウェアラブルデバイスの市場の拡大や活況を見据えながら、適切なサービスを開発するために、様々な企業との連携をさらに深めていく。

G．研究発表

1. 論文発表

該当なし

2. 学会発表

該当なし

H．知的財産権の出願・登録状況

該当なし

1. 特許取得

該当なし

2. 実用新案登録

該当なし

様式第１９

学 会 等 発 表 実 績

委託業務題目「常時測定・変動解析用カフなしウェアラブル血圧計の実用化を加速する臨床評価と無線遠隔システムへの応用」

機関名　独立行政法人 国立循環器病研究センター

１．学会等における口頭・ポスター発表

発表した成果（発表題目、口頭・ポスター発表の別）	発表者氏名	発表した場所（学会等名）	発表した時期	国内・外の別
How to submit your works - Various precaution you should have in mind before submission -　口頭	杉町　勝	第53回日本生体医工学会大会	2014 Jun	国内
迷走神経の電気刺激による急性心筋梗塞ラットの致死性不整脈死の制御及び心臓リモデリングの予防改善作用　口	李　梅花、稲垣　正司、鄭　燦、川田　徹、上村　和紀、杉町　勝	第53回日本生体医工学会大会	2014 Jun	国内
ラットにおける動脈圧受容器反射中枢弓の高域遮断特性　口頭	川田　徹、清水　秀二、李　梅花、鄭　燦、上村　和紀、神谷　厚範、杉町	第53回日本生体医工学会大会	2014 Jun	国内
ラットにおける迷走神経慢性電気刺激方法　口頭	鄭　燦、李　梅花、川田　徹、上村　和紀、稲垣　正司、杉町　勝	第53回日本生体医工学会大会	2014 Jun	国内
肺動脈楔入圧の、画期的な低侵襲推定法の開発　口頭	上村　和紀、稲垣　正司、鄭　燦、李　梅花、川田　徹、杉町　勝	第53回日本生体医工学会大会	2014 Jun	国内
交感神経活動と血中ノルアドレナリンの関係は直線的か？　口頭	川田　徹、清水　秀二、李　梅花、鄭　燦、ターナー　マイケルジェーム	第35回日本循環制御医学会総会	2014 Jul	国内
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下大静脈からの部分肺循環補助は、Fontan循環の血行動態を改善する　口	清水　秀二、川田　徹、杉町　勝	第35回日本循環制御医学会総会	2014 Jul	国内
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Application of acupuncture to circulatory regulation using engineering approach. 口頭	Kawada T, Sugimachi M.	ライフエンジニアリング部門シンポジウム2014	2014 Sep	国内
左心低形成症候群に対するハイブリッド手術の血行動態シミュレーション 口頭	清水 秀二、川田 徹、ターナー マイケル ジェームズ、宍戸 稔	第107回近畿生理学談話会	2014 Oct	国内
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掲載した論文（発表題目）	発表者氏名	発表した場所（学会誌・雑誌等名）	発表した時期	国内・外の別
Guanfacine enhances cardiac acetylcholine release with little effect on norepinephrine release in anesthetized rabbits.	Shimizu S, Kawada T, Akiyama T, Turner MJ, Shishido T, Kamiya A, Shirai M, Sugimachi M.	Auton Neurosci.	2015 Jan	国外
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（注１）発表者氏名は、連名による発表の場合には、筆頭者を先頭にして全員を記載すること。

（注２）本様式はexcel形式にて作成し、甲が求める場合は別途電子データを納入すること。

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Short communication

Guanfacine enhances cardiac acetylcholine release with little effect on norepinephrine release in anesthetized rabbits

Shuji Shimizu [a,*], Toru Kawada [a], Tsuyoshi Akiyama [b], Michael James Turner [a], Toshiaki Shishido [c], Atsunori Kamiya [a], Mikiyasu Shirai [b], Masaru Sugimachi [a]

[a] Department of Cardiovascular Dynamics, National Cerebral and Cardiovascular Center, Osaka 565-8565, Japan
[b] Department of Cardiac Physiology, National Cerebral and Cardiovascular Center, Osaka 565-8565, Japan
[c] Department of Research Promotion and Management, National Cerebral and Cardiovascular Center, Osaka 565-8565, Japan

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ABSTRACT

An α_{2A}-adrenergic agonist guanfacine improves autonomic imbalance in attention-deficit hyperactivity disorder, suggesting that it may be useful to correct autonomic imbalance in chronic heart failure (CHF) patients. To investigate the effects of guanfacine on cardiac autonomic nerve activities, a microdialysis technique was applied to anesthetized rabbit heart. Acetylcholine (ACh) and norepinephrine (NE) concentrations in atrial dialysates were measured as indices of cardiac autonomic nerve activities. Guanfacine at a dose of 100 μg/kg significantly decreased heart rate and increased dialysate ACh concentration without decreasing sympathetic NE release. Guanfacine may be useful for vagal activation therapy in CHF patients.

1. Introduction

Autonomic imbalance with activation of sympathetic nerve system and suppression of vagal nerve system causes progression of heart failure. Vagal activation has recently become a therapeutic option to correct autonomic imbalance in patients with chronic heart failure (CHF) (De Ferrari and Schwartz, 2011). Currently a clinical trial of electrical vagal nerve stimulation (VNS) for CHF is on-going (Hauptman et al., 2012). We have already demonstrated that an α_2-adrenergic agonist, medetomidine, activates cardiac vagal nerve (Shimizu et al., 2012), suggesting that a class of α_2-adrenergic agonists may correct the autonomic imbalance in CHF patients. However, medetomidine also has a sedative anesthetic effect. This may prevent widespread clinical use of medetomidine or dexmedetomidine in CHF treatment. Furthermore, severe hypotension during medetomidine treatment may also limit its clinical use.

Guanfacine, a selective α_{2A}-adrenergic agonist, has recently been approved for the treatment of attention-deficit hyperactivity disorder (ADHD) (Biederman et al., 2008). A systematic review suggests that children with unmedicated ADHD experience lower levels of cardiac vagal control than healthy controls, and guanfacine partly corrects this autonomic imbalance in ADHD patients (Rash and Aguirre-Camacho, 2012). Furthermore, Yamazaki et al. (2005) have reported that guanfacine improves sympathovagal imbalance related to rapid-eye-movement (REM)/non-REM ultradian sleep rhythm in CHF patients. Thus, guanfacine may be a potential pharmacological agent for vagal activation therapy in CHF patients. To clarify the effects of guanfacine on cardiac autonomic nerve activities, we applied a microdialysis technique to rabbit heart.

2. Materials and methods

2.1. Surgical preparation

Animal care was provided in accordance with the *Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences* published by the Physiological Society of Japan. All protocols were approved by the Animal Subject Committee of the National Cerebral and Cardiovascular Center. Seven Japanese white rabbits weighing 2.4 to 2.8 kg were used in this study. Anesthesia was initiated by an intravenous injection of pentobarbital sodium (50 mg/kg) via the marginal ear vein, and then maintained at an appropriate level by continuous intravenous infusion of α-chloralose and urethane (16 $mg \cdot kg^{-1} \cdot h^{-1}$ and 100 $mg \cdot kg^{-1} \cdot h^{-1}$, respectively). Adequate anesthesia level was confirmed by loss of the ear pinch response. The animals were ventilated mechanically with a mixture of room air and oxygen (respiratory rate, 30 cycles/min; volume, 15 ml/kg). A fluid-filled catheter was inserted

* Corresponding author at: Department of Cardiovascular Dynamics, National Cerebral and Cardiovascular Center, 5-7-1 Fujishiro-dai, Suita, Osaka 565-8565, Japan. Tel.: +81 6 6833 5012; fax: +81 6 6835 5403.
E-mail address: shujismz@ri.ncvc.go.jp (S. Shimizu).

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into the femoral artery to monitor systemic arterial pressure. Esophageal temperature was maintained between 38 and 39 °C using a heating pad.

With the animal in supine position, a right lateral thoracotomy was performed and the right 3rd to 5th ribs were partially resected to expose the heart. After pericardium incision, a dialysis probe was implanted as described in *Dialysis Technique* below. Three stainless steel electrodes were attached around the thoracotomy incision for monitoring body surface electrocardiogram (ECG). The ECG was connected to a cardiotachometer and heart rate was recorded.

At the end of the experiment, the animal was euthanized by injecting an overdose of pentobarbital sodium. In the postmortem examination, the inside of the resected atrial wall was observed macroscopically to confirm that the dialysis membrane was implanted totally within the atrial myocardium.

2.2. Dialysis technique

The materials and properties of the dialysis probe have been described previously (Shimizu et al., 2009, 2010). A dialysis fiber of semipermeable membrane (length 4 mm, PAN-1200; Asahi Chemical, Tokyo, Japan) was attached at both ends to polyethylene tubes (length 25 cm). The dialysis probe was implanted into the right atrial myocardium near the sinoatrial node, and was perfused with Ringer's solution containing a cholinesterase inhibitor, eserine (100 μM), at a speed of 2 μl/min using a microinjection pump (CMA/102, Carnegie Medicin, Sweden). Experimental protocol was started 2 h after implantation. Eight microliters of phosphate buffer (pH 3.5) was added to each sample tube before dialysate sampling, and each dialysate sampling period was set at 20 min (1 sample volume = 40 μl). Dialysate acetylcholine (ACh) and norepinephrine (NE) concentrations were analyzed separately by high performance liquid chromatography (Akiyama et al., 1991, 1994).

2.3. Experimental protocols

We investigated the effects of intravenous guanfacine on vagal ACh and sympathetic NE releases into the myocardium. Baseline dialysate samples were collected over 20 min before the injection of guanfacine. A low dose (10 μg/kg) of guanfacine (Sigma-Aldrich Co. LLC., St. Louis, MO, USA) was injected intravenously via the femoral vein. After approximately 20-min hemodynamic stabilization, dialysate was sampled for 20 min (40 μl). Thereafter, a high dose (100 μg/kg) of guanfacine was injected intravenously and another 20-min dialysate sample was collected after 20-min hemodynamic stabilization. Finally, bilateral cervical vagotomy was performed and a 20-min dialysate sample was collected 5 min after vagotomy taking into account the dead space between the dialysate membrane and the sample tube.

2.4. Statistical analysis

All data are presented as mean ± standard error. Heart rate and mean arterial pressure were compared by one-way repeated measures analysis of variance (ANOVA) followed by a Dunnett's test against baseline. After logarithmic transformation, dialysate ACh and NE concentrations were also compared by one-way repeated measures ANOVA followed by a Dunnett's test against baseline. Differences were considered significant at $P < 0.05$.

3. Results

Intravenous guanfacine at a dose of 10 μg/kg did not affect heart rate (264 ± 8 bpm at baseline to 243 ± 7 bpm, not significant) and mean arterial pressure (88 ± 2 mm Hg at baseline to 77 ± 2 mm Hg, not significant) (Fig. 1A and B). Dialysate ACh and NE concentrations at baseline were 6.7 ± 1.2 nM and 193 ± 22 pM, respectively (Fig. 2A and B). Intravenous injection of 10 μg/kg of guanfacine did not affect dialysate ACh and NE concentrations (7.3 ± 1.2 nM and 156 ± 23 pM, respectively).

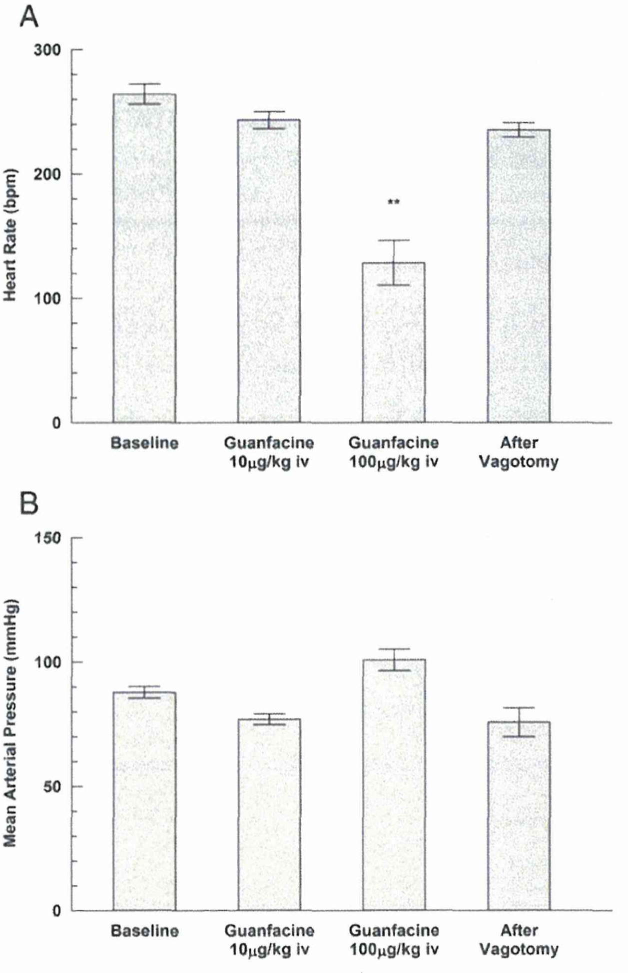

Fig. 1. Heart rate (A) and mean arterial pressure (B) at baseline, after intravenous injection (iv.) of guanfacine, and after bilateral cervical vagaotomy. **, $P < 0.01$ by Dunnett's test against baseline.

Intravenous guanfacine at a dose of 100 μg/kg significantly decreased heart rate to 128 ± 18 bpm ($P < 0.01$ vs. baseline), but had no effect on mean arterial pressure (101 ± 4 mm Hg, not significant vs. baseline) (Fig. 1B). Intravenous injection of 100 μg/kg of guanfacine significantly increased dialysate ACh concentration to 41.7 ± 8.4 nM ($P < 0.01$ vs. baseline) (Fig. 2A), whereas this dose of guanfacine did not affect dialysate NE concentration (172 ± 36 pM, not significant vs. baseline) (Fig. 2B). Heart rate and dialysate ACh concentration recovered to the baseline levels immediately after vagotomy (235 ± 6 bpm and 7.8 ± 1.2 nM, respectively).

4. Discussion

Guanfacine, a selective α_{2A}-adrenergic agonist, was previously used as a centrally acting antihypertensive drug because study indicated that guanfacine acted on the central nervous system and suppressed sympathetic nerve activity (Scholtysik, 1986). Although α_{2A}-adrenergic receptor subtype plays a principal role in central hypotensive effects of α_2-adrenergic agonists (MacMillan et al, 1996), the sympatholytic effect of guanfacine seems to be weaker than those of other α_2-adrenergic agonists. Our previous study demonstrated that 10 and 100 μg/kg of medetomidine, another α_2-adrenergic agonist, significantly decreased sympathetic NE release to the heart (Shimizu et al., 2012).

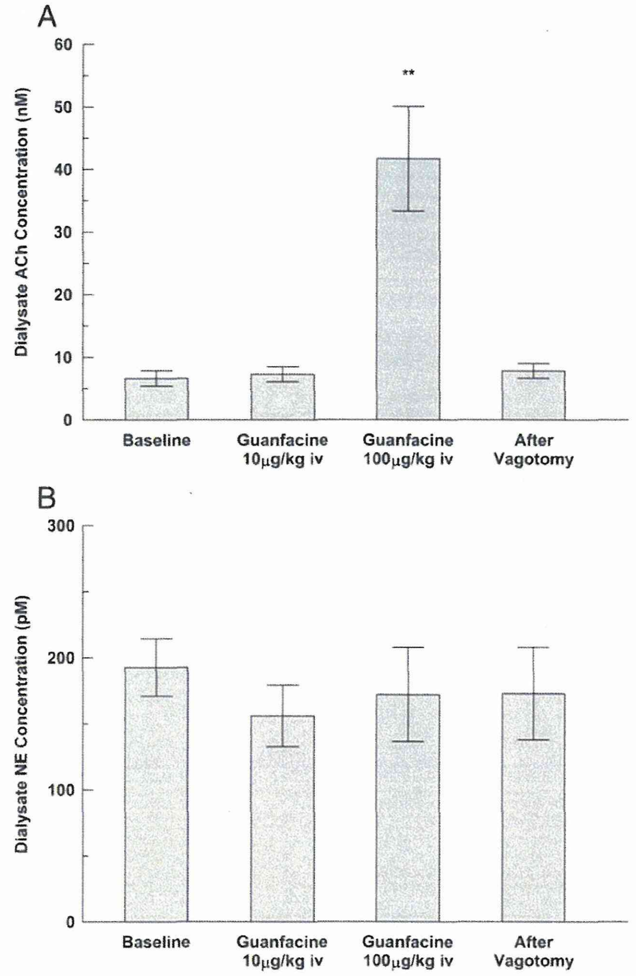

Fig. 2. Dialysate acetylcholine (ACh, A) and norepinephrine (NE, B) concentrations at baseline, after intravenous injection (iv.) of guanfacine and after bilateral cervical vagotomy. **, $P < 0.01$ by Dunnett's test against baseline.

In the present study, 10 μg/kg of guanfacine tended to decrease sympathetic NE release ($P = 0.08$), but this decrease did not reach a statistical significance. One-hundred microgram per kilogram of guanfacine did not affect sympathetic NE release. This little effect on sympathetic NE release may be due to the structure of guanfacine. Other α_2-adrenergic agonists such as medetomidine, dexmedetomidine and clonidine have an imidazole structure, and act on imidazoline receptors as well as α_2-adrenergic receptors. Recent study suggests that an imidazoline receptor agonist, moxonidine, centrally suppresses sympathetic nerve activity (Peng et al., 2009). Thus, the action of other α_2-adrenergic agonists on imidazoline receptors may contribute to the strong sympatholytic effect exhibited by these agents. On the other hand, guanfacine has no imidazole structure. Thus, the effect of guanfacine on sympathetic nerve activity may be totally dependent on its action on α_2-adrenergic receptor, which would account for the relatively weak effect. Although further investigations are necessary to explain the relatively weak effect on sympathetic nerve activity, this mechanism may be a reason why guanfacine is regarded as a second-line drug for hypertension, compared to other drugs such as calcium antagonists and angiotensin II receptor blockers (Sorkin and Heel, 1986).

The effect of guanfacine on vagal nerve activity has remained unclear. However, several studies suggest that α_2-adrenergic agonists may activate cardiac vagal nerve. Philbin et al. (2010) showed that clonidine significantly inhibited GABAergic neurotransmission to cardiac vagal neurons in the nucleus ambiguus. Inhibition of GABAergic neurotransmission may increase vagal activity to the heart. Kamibayashi et al. (1995) reported that the antidysrhythmic effect of dexmedetomidine was abolished in both vagotomized and atropine-treated dogs. Yamazaki et al. (2005) reported that guanfacine increased the power of high frequency component of heat rate variability during sleep. However, these findings are no more than indirect evidence that α_2-adrenergic agonists may activate cardiac vagal nerve. No direct evidence was available to confirm whether α_2-adrenergic agonists are able to activate cardiac vagal nerve, because it was difficult to selectively monitor cardiac vagal nerve activity in the past. Using a cardiac microdialysis technique, we have already reported that medetomidine, an α_2-adrenergic agonist, enhances vagal ACh release to the heart (Shimizu et al., 2012). Thus, the cardiac microdialysis technique may be the only method that allows selective monitoring of cardiac vagal nerve activity, apart from a single cardiac vagal fiber recording method reported previously (Cerati and Schwartz, 1991). In the present study using this technique, we demonstrated that 100 μg/kg of guanfacine increased vagal ACh release to the heart and this increase was abolished by bilateral cervical vagotomy. This result is direct evidence that guanfacine can activate cardiac vagal nerve. Since α_2-adrenergic receptors are known to be distributed in the nucleus tractus solitaries and nucleus ambiguus (Philbin et al., 2010; Robertson and Leslie, 1985), guanfacine may act on these nuclei to increase vagal ACh release to the heart.

The present study suggests that guanfacine has several advantages in various clinical settings, compared to other α_2-adrenergic agonists such as medetomidine. First, guanfacine causes less sedation than other α_2-adrenergic agonists (Scholtysik, 1986). Second, although guanfacine has been reported to cause hypotension, the changes in blood pressure are small to moderate and not clinically significant (Biederman et al., 2008). The dose of guanfacine (100 μg/kg) used in the present study is almost equivalent to the daily dose (80 to 120 μg/kg/day) for the treatment of ADHD in the clinical setting. However, this high dose of guanfacine did not cause severe hypotension. Thus, guanfacine may be a more favorable agent for vagal activation therapy in CHF patients compared to other α_2-adrenergic agonists.

This study has several methodological considerations. First, this experiment was performed under α-chloralose and urethane anesthesia. Because chloralose–urethane anesthesia reduced cardiac vagal efferent activity (Korner et al., 1968), vagotonic effect of guanfacine might have been more easily demonstrated compared with conscious conditions. On the other hand, we have already reported that an α_2-adrenergic agonist, medetomidine, enhances vagal ACh release through the modulation of baroreflex (Shimizu et al., 2012). Therefore, we think that vagal activation of guanfacine may be a direct action to the central nervous system. However, further investigations are necessary to clarify the mechanism of guanfacine-induced vagal activation. Second, the dose-dependent response of guanfacine was not examined in random order because plasma half-life of guanfacine was reported to be over 2 h (Barber and Reid, 1982). Therefore, 10 μg/kg of guanfacine might have partly affected the results of 100 μg/kg of guanfacine.

In conclusion, intravenous guanfacine at a dose of 100 μg/kg significantly enhanced vagal ACh release to the heart with no significant effect on sympathetic NE release. This vagotonic effect of guanfacine may be beneficial for vagal activation therapy in CHF patients.

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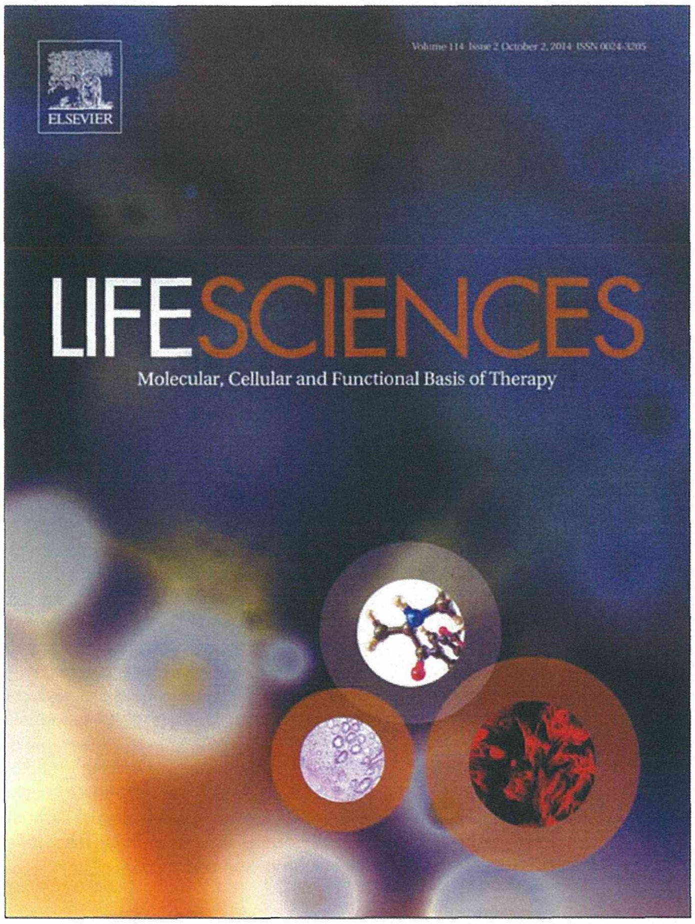
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