Perhexiline Therapy in Patients with Type 2 Diabetes: Dissociation Between Potentiation of Nitric Oxide Signalling and Changes in Insulin Resistance

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Abstract

Purpose: Perhexiline (Px) has previously been utilized primarily in the treatment of otherwise refractory angina pectoris and/or systolic heart failure. In recent years, Px has also shown increasing promise as a potential chemotherapeutic agent. Px inhibits carnitine palmitoyltransferases 1 and 2 (CPT1, CPT2), which control uptake of long-chain fatty acids into mitochondria and thus represent the rate-limiting steps in their metabolism. However, occasional cases of hypoglycaemia have been reported in Px-treated patients, raising the possibility that Px may also increase sensitivity to insulin. Furthermore, Px increases anti-aggregatory responses to nitric oxide (NO), an effect which may parallel insulin sensitization. No previous studies have examined either the effect of Px on insulin sensitivity, or the relationship of such putative changes with effects on NO signalling. We therefore sought to examine these relationships in patients with stable T2D and cardiovascular disease.

Methods: In 30 patients with concomitant T2D and cardiovascular disease, Px was initiated, and dosage was titrated to reach therapeutic range and to prevent toxicity. Investigations were performed before and after 2 weeks, to examine changes in insulin sensitivity, platelet responsiveness to the anti-aggregatory effects of the NO donor sodium nitroprusside (SNP), as well as other markers of inflammation and modulators of NO signaling.

Results: Px substantially potentiated inhibition of ADP-induced platelet aggregation by SNP (from 16.7±3.0 to 27.3±3.7%; p=0.005). Px did not change fasting blood glucose concentrations and reduced insulin sensitivity (HOMA-IR score increased from median of 4.47 to 6.08; p=0.028), via increasing fasting plasma insulin concentrations (median 16.5 to 19.0 mU/L: p=0.014). Increases in SNP responses tended (r=-0.30; p=0.11) to be reciprocally related to increases in HOMA-IR. No patient developed symptomatic hypoglycaemia, nor was there any other short-term toxicity of Px.

Conclusions: In patients with stable T2D and cardiovascular disease, Px is not an insulin sensitizer, and does not normally induce hypoglycaemia. These results effectively dissociate the NO-sensitizing effect of Px from variability in insulin signaling.

Introduction

Type 2 diabetes (T2D) is an increasingly prevalent problem throughout the world and is associated with a substantial increase in prevalence of both stable myocardial ischaemia and heart failure, as well as of both the incidence of acute myocardial infarction and associated mortality risk. Furthermore, patients with T2D are at increased risk for carcinogenesis [1].

These adverse prognostic aspects of T2D have contributed to investigations to identify biochemical modulators of cardiovascular risk, as well of the nexus between impaired responsiveness to insulin and propensity towards both myocardial ischaemia and development of cancer. It has been shown that extents of both insulin resistance [2] and of hyperglycaemia, especially at times of clinical crises [3], represent adverse prognostic markers. Furthermore, severe hyperglycaemia represents a basis for

increased mitochondrial formation of superoxide anion (O_2-), a major mediator of many of the cardiovascular complications of diabetes [4], with resultant "scavenging" of nitric oxide (NO) and therefore impairment of its vasodilator and anti-aggregatory effects, inducing “NO resistance”. NO resistance, represents an adverse prognostic marker [5], whether measured via the coronary vasodilator [6] or the anti-aggregatory [5] effects of NO.

Insulin infusion [7], the ACE inhibitor ramipril [8], the hydrogen sulphide donor N-acetylcysteine [9] and the prophylactic anti-anginal agent perhexiline (Px) [10] have all been shown to attenuate NO resistance, although the precise mechanism(s) underlying this effect have never been fully defined.

In the case of Px, both its impact on insulin sensitivity in patients with diabetes and its effects on maintenance of homeostasis at the platelet level are issues of increasing importance. The range of clinical utility of Px has expanded considerably, following demonstration that its potential long-term hepatotoxicity and neurotoxicity can be prevented by maintenance of plasma Px concentrations within a defined therapeutic range [11, 12] and that Px is safe for patients with cardiac and renal insufficiency [13].

Px is now recognised as inducing a “metabolic” prophylactic antianginal effect, with its major mechanism of action identified as induction of a cardiac metabolic shift from long-chain fatty acid to glucose oxidation via inhibition of the rate-limiting enzyme carnitine palmitoyltransferase-1 (CPT-1), and to a lesser extent CPT-2 [14]. Therefore, in theory, Px should activate a “Randle Shift” [15], whereby there is a compensatory increase in glucose utilization when fatty acid utilization is suppressed. In theory, this adjustment of substrate utilization will lead to an increase in efficiency of cardiac oxygen utilization [14, 16, 17]. These effects have opened up new therapeutic options for Px, which include the management of systolic heart failure [13, 17] and non-obstructive hypertrophic cardiomyopathy [18].

Recently, several preclinical studies have suggested that Px also exerts substantial antineoplastic effects, both in tumour cell lines and in vivo [19–23]. The concept of an agent with combined antineoplastic and cardioprotective effects is very attractive on a theoretical basis. However, to date, there has been little evidence that effecting a Randle shift in cardiac metabolism, representing a means for maintaining cellular energetics despite reduced substrate availability, might interact directly either with the actions of insulin (in promoting cellular uptake of glucose) or the Warburg effect (of activating anaerobic metabolism in association with inappropriate cellular proliferation).

The introduction of Px into the therapeutic arena preceded the utilization of therapeutic drug monitoring. Several cases of hypoglycaemia, sometimes severe, were reported in the early literature [24–27]. However, no detailed studies of Px effects on insulin signalling have been reported to date. The currently reported study was therefore undertaken to evaluate the effects of Px therapy on insulin responsiveness in patients with stable T2D and cardiovascular disease, and to determine whether its effects on insulin signalling might parallel changes in tissue responsiveness to NO.

Methods

Patient selection

Adult patients with T2D were considered for inclusion if they were concurrently under consideration for the initiation of Px treatment for the management of various cardiovascular disease states. These include refractory angina pectoris, systolic heart failure, or symptomatic aortic valve stenosis. Exclusion criteria were (1) current or potential pregnancy, (2) concurrent therapy with any $P2Y_{12}$ receptor antagonist (which would obscure effects on planned platelet aggregation studies), or (3) previous adverse effect of Px. The protocol was approved by the local Ethics of Human Research Committee (HREC/13/TQEHLMH/220) and registered in the Australian and New Zealand Clinical Trials Registry (ACTRN126150004297505). Informed consent for participation and publication of results was obtained from participants prior to entry.

Study design

The study was designed as a comparison of the effects of two weeks' treatment with Px on (i) insulin sensitivity in patients with well-controlled Type 2 diabetes mellitus (primary endpoint) and (ii) platelet responsiveness to the anti-aggregatory effects of nitric oxide.

Following baseline evaluations, Px treatment was initiated with a rapid loading regimen [28] of 600mg on the first day, followed by adjustment of dosage on the basis of initial plasma concentrations of Px and its monohydroxylated metabolite [28]. Plasma Px concentrations were re-assayed after 2 weeks' of treatment.

Investigations

The following were performed before initiation of Px, and at the end of the study period. Patients were advised to fast overnight, and blood samples were drawn into acid citrate anticoagulant, on the following morning between 0800 to 0900. Investigations performed included:

1. Determination of fasting blood glucose concentrations and plasma insulin concentrations to measure insulin resistance as HOMA-IR, representing the primary endpoint, and insulin sensitivity by QUICKI score.
2. Measurement of platelet pro-aggregatory responses to ADP and anti-aggregatory responses to the NO donor sodium nitroprusside (SNP). Whole blood impedance aggregometry (Model 560, Chrono-Log, Haverstown, Pennsylvania) was used to record platelet aggregation, in Ohms [29]. Blood samples were stirred at 900rpm at 37ºC, and platelet aggregation was induced by 2.5μM ADP; inhibition of aggregation was induced by 10μM of SNP.
3. The putative effects of Px on markers of inflammation and modulators of NO signalling were also determined. These included plasma concentrations of thrombospondin-1 (TSP-1), which can inhibit soluble guanylate cyclase activation [30] (assayed with ELISA kit, R&D systems, MN, USA); asymmetric dimethylarginine, a competitive inhibitor of NO synthase was assayed using previously published HPLC assay [31, 32]; and myeloperoxidase (MPO), a source of release of hypochlorous

acid from neutrophils (ELISA kit, Mercodia, Sweden) [33]. Platelet content of thioredoxin-interacting protein (TXNIP), an inflammatory activator [34], was also determined [35].

Statistical methodology

The study results were assessed based on intention-to-treat principles and the limit of statistical significance was taken as $p<0.05$. All parameters were compared on a paired basis before and after 2 weeks' Px therapy, using either Student's paired t-test or a paired Wilcoxon test as appropriate. The inclusion of 30 patients ensured a power of the primary endpoint (insulin resistance measured by HOMA-IR) of $\alpha = 0.05$, $\beta = 0.80$ to detect a 0.5 SD fluctuation post Px. Correlations between Px effects on HOMA-IR and SNP response were sought using Pearson's correlation coefficient. Data are expressed as mean ± SEM unless otherwise stated.

Results

Clinical data related to the participants are summarised in Table 1. In general, this was an ageing group of patients with well-controlled T2D and mild to moderate renal impairment. The most common indication for Px therapy was refractory angina pectoris. These patients were still symptomatic prior to initiation of Px therapy despite receiving at least one long-acting prophylactic anti-anginal agent. Other indications for Px therapy include symptomatic heart failure [16, 17] and aortic stenosis [36]. Most patients were also receiving either ACE inhibitors or angiotensin receptor blocker therapy. Regarding treatment for T2D, most patients received more than one therapy, and metformin remained the most commonly utilized oral hypoglycaemia agent.

Table 1
Baseline clinical characteristics (n=30)

Patient characteristics	
Age (years)	70 ± 2.2
Female (%)	37
HbA1c (%)	7.3 ± 0.26
Baseline serum creatinine (μmol/L)	112.7 ± 13.74
Major indication(s) for Perhexiline therapy	
Refractory angina (%)	70
Systolic heart failure (%)	23
Symptomatic aortic stenosis (%)	7
Concurrent pharmacotherapy	
ACE-inhibitor/ ARB (%)	73
Calcium channel antagonist (%)	43
Beta-blocker (%)	37
Nitrate (%)	40
Metformin (%)	60
Insulin (%)	33
Sulphonylurea (%)	30
DPP-IV inhibitor or thiazolidinedione (%)	20

No patient developed symptomatic adverse effects from Px therapy over the two weeks duration of the study. Three patients required major reductions in dosage because of CYP2D6 poor metaboliser phenotype [37]. Median plasma Px concentration after two weeks' treatment was 0.26 (0.25,0.43) mg/L (therapeutic range 0.15 to 0.60 mg/L) [11, 38].

Table 2 summarises effects of Px on parameters of insulin secretion and of tissue responsiveness to insulin. HOMA-IR score, the primary endpoint of the study, increased significantly post Px treatment, indicating accentuation of insulin resistance. This change reflected an approximately 13% increase in plasma insulin concentrations, without significant change in fasting blood glucose levels. However, no patient experienced any symptomatic hypoglycaemic episodes.

Table 2
Effects of two weeks of Perhexiline therapy on blood glucose, insulin concentrations and insulin sensitivity scores.

Parameters	Before Px	After Px	p value
Blood glucose level (mmol/L)	6.8 (5.7, 8.6)	7.0 (6.0, 8.7)	0.37
Plasma insulin level (mU/L)	16.5 (11.8, 25.3)	19.0 (11.8, 37.3)	<0.05
HOMA-IR score	4.47 (3.42, 8.55)	6.15 (3.05, 15.06)	<0.05
QUICKI score	0.158 ± 0.001	0.156 ± 0.002	0.07

As previously reported [10], Px therapy did not significantly affect extent of ADP-induced platelet aggregation (Fig. 1A), but potentiated anti-aggregatory effects of the NO donor SNP (Figure 1B).

To determine whether changes in HOMA-IR values in individual patients also related to increases in NO sensitivity induced by Px, correlations were sought between proportional change in HOMA-IR and in SNP response in individual patients. The results, shown in Figure 2, indicate that there was no suggestion of a direct relationship: in fact, increases in NO response tended (p=0.11) to be most marked in patients in whom there was no induction of insulin resistance.

Platelet content of TXNIP and plasma concentrations of ADMA, MPO and TSP-1 did not change significantly under treatment with Px (Figure 3).

Discussion

Px has long been established as a potent prophylactic anti-anginal agent, whether used as monotherapy or in combination with other drugs [38–40]. It has been shown that Px improves symptomatic status and left ventricular systolic function in patients with systolic heart failure [17], as well as cardiac energetics in patients with dilated and hypertrophic cardiomyopathy [16, 18]. Finally, recent preclinical studies have established the potential utility of Px in the treatment of malignancies, both as a sensitizer to chemotherapy or as a tumour-suppressive agent [20–23].

The main residual barrier to the widespread use of Px in the treatment both of cardiovascular disease and of malignancy is therefore the potential for induction of hepato- and neurotoxicity. However, the potential for Px to induce hepatitis and/or peripheral neuropathy during long-term therapy has been dramatically reduced by the availability of therapeutic drug monitoring of plasma concentrations of Px and of its hydroxylated metabolites [12, 41, 42]. This led to the only remaining concern being the risk of hypoglycaemia, which has been reported as a rare but potentially serious adverse effect in some case reports [27, 43, 44], even though the cause of hypoglycaemia remained uncertain. Therefore, the primary objective of the current study was to determine whether induction of hypoglycaemia remains a significant problem when Px is utilized for treatment of heart disease in patients with diabetes.

The results of the study indicate that short-term Px therapy, titrated to achieve therapeutic plasma Px concentrations, does not affect fasting blood glucose concentrations, while significantly increasing plasma insulin concentrations. On this basis, Px technically increased insulin resistance, as measured by HOMA-IR. Furthermore, consistent with previous observations in patients with severe angina pectoris, Px normalises anti-aggregatory responses to the NO donor SNP [10], and thus ameliorates “NO resistance”, a condition known to be an independent negative prognostic marker [5, 6]. This is an important finding, especially in patients with diabetes, as they are at increased risk of adverse outcomes in the presence of acute myocardial ischaemia or heart failure.

To test the hypothesis that the impact of Px on HOMA-IR and platelet responsiveness to NO reflects a common mechanistic pathway, we sought evidence of correlation between these parameters. While there was no significant relationship, the two parameters tended to have an inverse, rather than direct correlation. Thus it seems that these two Px effects reflect different mechanisms: *NO sensitization by Px does not result from stimulation of insulin release*, despite our previous finding that insulin infusion, administered to patients to correct hyperglycaemia, also reverses NO resistance [7].

As originally proposed by Randle et al [15], fatty acids and glucose compete for selection and oxidation by muscles and adipose tissues. Therefore, inhibition of fatty acid metabolism induces a shift towards glucose utilization, potentially mediating increases in cardiac metabolic efficiency. If glucose utilization were increased simultaneously with glucose uptake into tissues such as muscle, this could potentially induce hypoglycaemia. However, in many circumstances, especially during the fed state, insulin effects on tissue uptake of glucose are primarily associated with increased glycogen synthesis, rather than glucose utilization [45]. Therefore, increased plasma concentrations of insulin in the presence of Px do not always imply increased oxidation of glucose: it may well be that insulin secretion is not in any way a mediator of the “Randle Cycle”. Indeed, previous studies have suggested a dissociation of insulin signalling from substrate utilization [15, 46].

Although the mechanism is unclear, Px can increase plasma insulin concentrations potentially through its CPT-1 inhibition at the pancreatic islet beta-cells. It was previously demonstrated that the sulphonylurea glibenclamide inhibited CPT-1 in islet cells in a K_{ATP}-independent manner, as did another CPT-1 inhibitor, etomoxir, thereby stimulating the exocytosis of insulin [47]. Px may well exert a similar effect.

The study has some limitations. First, it is entirely possible, given the results, that risk of hypoglycaemia with Px may be greater in non-diabetic patients, given integrity of glucose uptake mechanisms, but this remains to be explored. We also do not know whether hyperinsulinaemia as a driver of insulin resistance carries adverse prognostic implications in the long-term. A larger sample size with longer duration of investigations would be necessary to evaluate this possibility. Finally, we do not yet understand the extent to which these findings are relevant to the emerging role of Px as an antineoplastic agent, but would emphasise that in this circumstance, the dependency of many cancers on CPT-modulated fatty acid uptake is likely to be a key mechanism of Px action.

In conclusion, in stable patients with T2D, short-term treatment with Px does not induce changes in fasting blood glucose levels, increases plasma insulin concentrations and sensitizes platelets to the anti-aggregatory effects of NO. The two latter effects are mechanistically disparate.

Declarations

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Data availability

Request to original data should be addressed to the corresponding author and may be made available according to local procedures.

Statements & Declarations

Funding & Competing Interests

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Author contributions

C-RC, YYC and JDH contributed to the study conception and design; YYC and JDH supervised the study programme; C-RC, SL, HI and TH contributed to data collection and analysis. The first draft of manuscript was written by C-RC and all authors commented on previous versions, the final version of the submitted manuscript was approved by all authors.

Ethics approval

The current study was performed in line with the ethical standards of the institutional research committee and the principles of the Declaration of Helsinki. Approval was granted by the Human Research Ethics Committee of The Queen Elizabeth Hospital (HREC/13/TQEHLMH/220).

Consent to Participate

All participants provided informed written consent.

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Figures

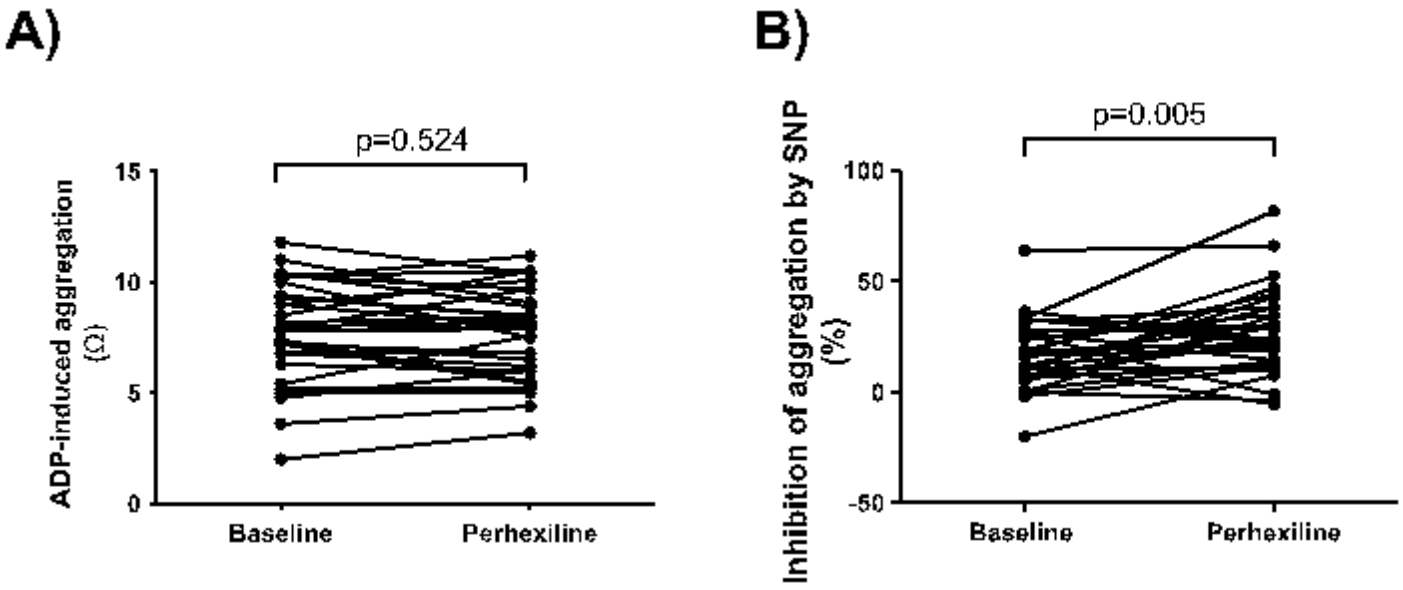

Figure 1

Effects of Perhexiline treatment on (A) ADP-induced platelet aggregation, (B) inhibition of ADP-induced platelet aggregation by sodium nitroprusside (SNP).

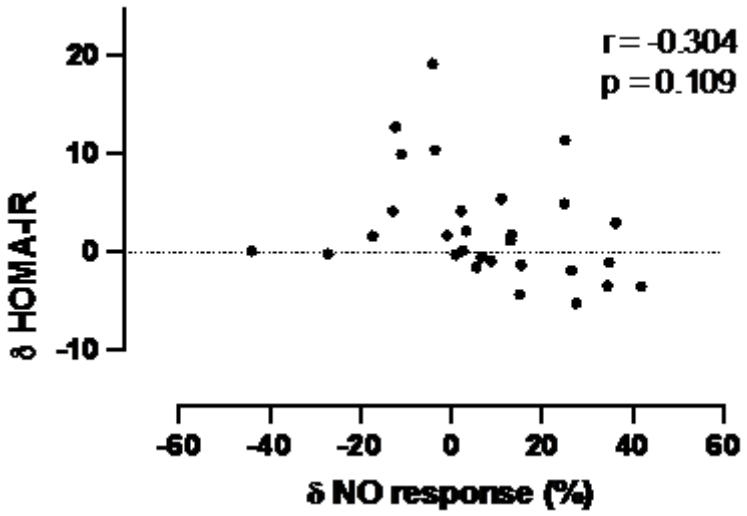

Figure 2

Correlation between changes in responses to nitric oxide and insulin sensitivity in patients treated with Perhexiline.

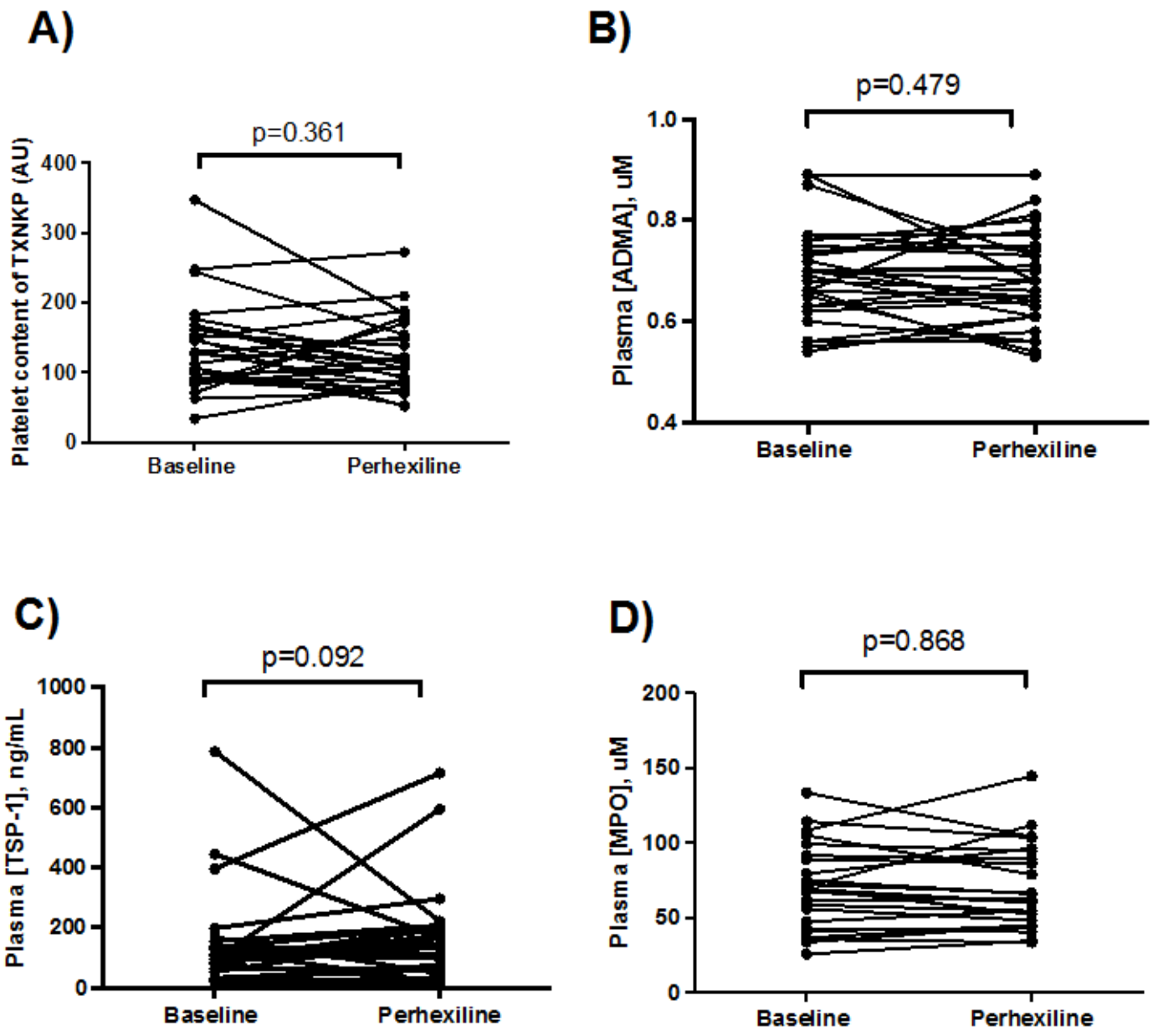

Figure 3

The effect of Perhexiline on platelet content of TXNIP, plasma concentrations of ADMA, TSP-1 and MPO.