Short-term effect of folic acid supplementation in renal transplant recipients and chronic kidney disease patients with comparable renal function impairment

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Résumé • Summary

Des données indirectes récentes suggèrent que l’efficacité de la supplémentation pharmacologique en acide folique pour réduire l’hyperhomocystéinémie serait équivalente chez les transplantés rénaux et chez les patients insuffisants rénaux chroniques (IRC) présentant un degré comparable de réduction de la fonction rénale. Toutefois, la comparaison directe de la réponse à la supplémentation en acide folique dans les deux populations n’a jamais été étudiée.

C’est pourquoi l’objectif de cette étude a été d’évaluer la réponse à l’acide folique (5 mg/jour) chez quinze transplantés rénaux ayant une néphropathie chronique du greffon, et chez quinze patients IRC (stade 3). Les patients des deux groupes vivants dans la région de Skopje (Macédoine) étaient comparables en âge, en sexe et en fonction rénale.

Après douze semaines de supplémentation en acide folique, une diminution significative des concentrations plasmatiques d’homocystéine a été observée chez les transplantés rénaux et chez les patients IRC. Le pourcentage de la réduction des taux plasmatiques d’homocystéine était identique dans les deux groupes (25,7% versus, 24,5%, p = NS). Ces résultats prouvent directement pour la première fois l’efficacité identique du traitement par l’acide folique dans les deux groupes de patients et suggèrent le rôle prépondérant de l’urémie dans la genèse de l’hyperhomocystéinémie.


Key words: Folic acid – Homocysteine – Chronic kidney disease – Renal transplant recipient.

Introduction

Despite major recent advances in renal transplantation there is increasing concern about cardiovascular events with inherent consequences for long-term patients and graft survival. Classical cardiovascular risk factors in general population such as hypertension, hyperlipidemia, diabetes mellitus, and cigarette smoking are common after renal transplantation, and they are effectively

Abbreviations
Hcy: Homocysteine

CKD: Chronic kidney disease
RTR: Renal transplant recipient
associated with the development of cardiovascular disease. It should be noted however, that although the classical cardiovascular risk factors predict cardiovascular disease after renal transplantation, they tend to underestimate the risk, especially the one associated with diabetes mellitus. Therefore, additional non-classical cardiovascular risk factors present in renal transplant population including hyperhomocysteinemia probably play a role in the genesis of cardiovascular disease, as well.

The plasma concentration of fasting total homocysteine (Hcy) is moderately elevated in renal transplant recipients, and this elevation is associated with an increased risk of cardiovascular disease in such patients. Renal function appears to be a particularly crucial determinant of plasma total Hcy levels in these patients. In a recent report, no difference in total Hcy concentration was found between renal transplant recipients (RTR) on standard immunosuppressive therapy and chronic kidney disease (CKD) patients with equivalent reduction of renal function but not treated by immunosuppressive drugs.

Supra-physiological folic acid-based supplementation was shown to decrease total Hcy concentrations in 50% of RTR. This high percentage of renal transplant recipient responders is similar to the percentage of responders in CKD patients. These findings suggest that RTR and CKD patients share common disturbances, mediated through residual renal function reduction. However, a direct comparison between RTR and CKD patients regarding their response to high dose folic acid supplementation has not yet been assessed. Our working hypothesis was that the efficacy of folic acid supplementation in reducing plasma total Hcy concentrations might be similar in RTR and CKD patients with comparable reduction of renal function.

### Patients and methods

Fifteen stable RTR with evidence of chronic allograft nephropathy, and fifteen CKD (stage 3) patients matched for age, sex and renal function, living in area of Skopje, Macedonia, and who had no vitamin B supplementation, agreed to participate at the study (Table I). RTR were under standard triple immunosuppressive therapy (Azathioprine or Mycophenolate mofetil, corticosteroids and Cyclosporin A). RTR and CKD patients were treated by folic acid (5 mg/day) for 12 weeks, and followed on an outpatient basis.

Blood samples were drawn after 12 h fasting from antecubital vein into Na-EDTA containing tubes, and centrifuged for 10 min at 3000 g. The plasma fractions were transferred to plastic vials and stored at –80°C for up to 12 months.

Plasma total Hcy levels were assessed in both groups before and after 12 weeks of folic acid supplementation by microplate enzyme immunoassay method (Bio Rad Laboratories) according to Frantzen et al. The total plasma levels of B12 and folic acid were assessed by the method of electric chemiluminescence (at wave length 620 nm) using automatic immuno-analyzer (Elcsys 2010, Roche Laboratories). Normal values for the Skopje region are serum vitamin B12, 184-505 pg/ml and serum folate, 2.63-8.79 ng/ml.

Results have been expressed as means ± SD. Difference between the groups was assessed using non-paired and paired Student’s t test for independent and dependent samples. Correlations between numeric parameters were assessed using Spearman rank order correlations.

### Results

Before folic acid supplementation, plasma total Hcy concentrations higher than 15 (i.e. above normal range) were present in 80% of RTR with chronic allograft nephropathy, as well as in CKD patients with a similar degree of renal impairment. The two groups had no vitamin B supplementation before the study, which may explain the high baseline levels of total Hcy observed in the present study. Although baseline plasma total Hcy levels were slightly higher in RTR than in CKD patients, the difference did not reach the level of significance (Table II). Of note, baseline serum folate levels were significantly lower in RTR than in CKD patients (Table II). Using Spearman rank order correlations, plasma folate (r = -0.54, p < 0.05), but not plasma albumin was negatively correlated with plasma total Hcy at baseline.

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<th>Tableau II: Baseline plasma biochemistry.</th>
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<tr>
<td>Total homocysteine (µM)</td>
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<td>Serum folate (ng/ml)</td>
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<td>Serum B12 (pg/ml)</td>
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<td>Serum albumin (g/l)</td>
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After 12 weeks of folic acid supplementation, plasma total Hcy concentrations were significantly reduced in the two groups (Table III). Percent reduction of plasma total Hcy levels was nearly identical in the two groups (25.7 % vs 24.5 %, p = NS).
**Tableau III:** Plasma total homocysteine and folate levels before and after folic acid supplementation for 12 weeks.

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<tr>
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<th>Renal transplant recipients (n = 15)</th>
<th>Chronic kidney disease patients (n = 15)</th>
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<tr>
<td></td>
<td>Baseline</td>
<td>After folic acid supplementation</td>
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<tr>
<td>Total homocysteine (µM)</td>
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<td></td>
<td>32.6 ± 5.1</td>
<td>24.2 ± 7.7*</td>
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<tr>
<td>Serum folate (ng/ml)</td>
<td>2.6 ± 0.3</td>
<td>19.2 ± 1.9*</td>
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*p < 0.01 (baseline vs post folic acid supplementation); RTR: renal transplant recipient; CKD: chronic kidney disease.

### Discussion

The results of this study show that the efficacy of folic acid supplementation in reducing plasma total Hcy concentration was similar in RTR and CKD patients with comparable renal function. Our results confirm previous observations regarding the efficacy of folic acid therapy given separately to either RTR or CKD patients, and extend them by direct confirmation of identical efficacy. Moreover, they are in favor of a lack of influence of immunosuppressive therapies on total Hcy concentrations in renal transplant patients, as previously suggested by epidemiological studies. It has been shown that long-term treatment with calcineurin inhibitors such as Cyclosporin A-microemulsion and Tacrolimus contributes to increased cardiovascular morbidity and mortality in RTR by mechanisms independent of the Hcy pathway. As mentioned above all transplant patients in the present study were on CyA. Therefore, we were not able to determine a possible role of immunosuppression therapy on total Hcy level. However, numerous studies (except one) failed to demonstrate a relationship between total Hcy levels and cyclosporine.

Folate supplementation failed to normalize plasma total Hcy levels in the majority of the patients of the present study. This failure might be due to lack of vitamin B6 and B12 supplementation, since the normalization of plasma total Hcy concentrations in both RTR and CKD patients has been observed with folic acid treatment associated with vitamin B6 and B12 supplementation.

Elevated plasma concentrations of fasting total Hcy are associated with an increased risk of cardiovascular disease in CKD patients. Hyperhomocysteinemia in patients with impaired renal function, both RTR and in CKD patients, may enhance vascular smooth muscle cell proliferation, increase platelet aggregation, and act on the coagulation cascade and fibrinolysis creating a prothrombotic environment. However, a synergistic effect of Hcy with other well-known risk factors in RTR remains possible. In a recent study, the pharmacological correction of hyperhomocysteinemia had a beneficial effect on carotid intima-media thickness in RTR. Moreover, the NIDDK recently allocated funds for a randomized, controlled Hcy-lowering trial designed to measure cardiovascular outcomes in 4000 RTR over a 5-year period.

The results of the present study are of importance, since if the NIDDK trial shows that lowering total Hcy levels by vitamin B supplementation leads to reduction in cardiovascular events, such findings could be directly applicable to CKD patients as well.