D-乳酸在糖尿病腎臟中之研究

The Study of D-Lactate in Diabetic Rat Kidney

中文摘要

動物體內僅有肝臟與腎臟可進行糖質新生的作用，在糖尿病下，腎臟糖質新生的增加使葡萄糖的釋放大量增加近 300%，被認爲是造成高血糖的主要原因。乳酸爲腎臟糖質新生的主要來源，其含有一不對稱碳，故具有 D-、L-乳酸兩種鏡相異構物，而 D-、L-乳酸兩者之生成相當不同，L-乳酸是糖解作用之終產物，D-乳酸爲體內一醣化終產物 (advanced glycation end-products) —甲基乙二醛 (methylglyoxal) 進行去毒化反應所生成，目前缺乏對乳酸鏡像異構物與腎臟糖質新生間相關的探討。

爲瞭解 D-乳酸在糖尿病腎臟中之含量，以及對於糖質新生的影響，故利用已建立之管柱切換高效液相層析法，檢測腎臟均質液中 D-乳酸之濃度，並利用一葡萄糖試劑組套，探討腎臟均質液中額外加入之 D-、L-乳酸對葡萄糖釋放的影響，以及腎臟中 D-乳酸與自由基的關係。

實驗結果顯示，以 HPLC 分析糖尿病大鼠腎臟中之 D-乳酸，發現會隨著誘導時間 1、2、3、4 個月而累積，且呈現一線性增加 (2.99, 13.11, 18.19, 23.23 mol/mg vs. 0.79 mol/mg as control groups)；在糖質新生方面，於腎臟均質液中添加之 D-乳酸 (3.47 g/ml) 可抑制 L-乳酸所釋放之葡萄糖(17.24 g/ml)，推測 D-乳酸在糖質新生作用上可與 L-乳酸拮抗；同時利用能捕捉自由基之活性氧原子的細胞色素 c (cytochrome c)，檢測含有外加 D-乳酸之腎臟均質液與控制組相較，發現有大量自由基的生成 (145% vs. 100% as control)，進而推論糖尿病中，堆積在腎臟無法排除之 D-乳酸會使氧壓 (oxidative stress) 增加，漸進對腎臟造成傷害，造成糖尿病的腎性病變 (diabetic nephropathy)。

英文摘要

Renal gluconeogenesis has been stressed in diabetes mellitus, as excess glucose is released into the circulation and hyperglycemia is intensified. The main gluconeogenic precursor in kidney is thought to be lactate; however, less is emphasized enantiomerically. L-lactate is a glycolysis end-product, but D-lactate is formed after detoxification of methylglyoxal, which is the main source of advanced glycation end-products.

For investigating the complete metabolism and the physiological role of D-lactate, we measured D-lactate levels in normal and diabetic rat kidney homogenates by our established column-switching HPLC method with fluorescence detection. The influence of D-lactate on gluconeogenesis was also estimated by determining the glucose concentrations in D- or L-lactate added in kidney homogenates. Furthermore,

the relation between D-lactate and free radicals was determined by cytochrome c with a UV spectrophotometer at 550 nm.

This study indicated that D-lactate concentrations in rat kidney were significantly and time-dependently accumulated in diabetic groups after induced for 1, 2, 3, 4 months (2.99, 13.11, 18.19, 23.23 mol/mg, respectively), as comparing that in normal groups (0.79 mol/mg). In addition, the histology of induced 3-month diabetic rat renal showed some structural changes of progressive diabetic nephropathy. Moreover, 80% of glucose released by addition of 6.0 mM of L-lactate (17.24 g/ml) was suppressed when 6.0 mM of D-lactate (3.47 g/ml) was supplied into rat kidney homogenates. It was supposed that D-lactate inhibited gluconeogenesis as an antagonist of L-lactate in rat kidney. On the other hand, the accumulation of D-lactate maybe damage the renal by generating the reactive oxygen species, in which it was determined by cytochrome c with external 6.0 mM D-lactate in rat kidney homogenates.