Real time detection of nitric oxide in the blood of rats following renal ischemia by ESR spectroscopy

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INTRODUCTION

The interest in nitric oxide (NO) free radical has grown with the discovery that it is involved in both normal homeostasis and pathophysiological processes, but the protective or deleterious effects it plays in ischemia/reperfusion (I/R) injury are still paradoxical and controversial. This is mostly due to the difficulties in direct and *in vivo* measurements of the NO free radical [1]. As electron spin resonance (ESR) spin trapping is an effective technique to directly reveal free radicals, we attempted in this study to use ESR spectroscopy to carry out an *in vivo* real time monitor of postischaemic NO level on rat renal ischemia/reperfusion model. Since when general dithiocarbamates are used as spin trap agents the formed spin adducts will degenerate in living body after a certain period, which causes the real time monitor very difficult, the endogenous hemoglobin in venous blood was employed as a natural spin-trap for NO in this study. Hemoglobin preferentially binds to NO forming relatively stable HbNO adduct (nitrosyl hemoglobin) which can be identified by a characteristic triplet ESR spectrum [2].

METHODS

6 male adult SD rats were subjected to anesthesia and laparotomy, then ischemia was initiated by clamping left renal artery. After 1 h ischemia, reperfusion was allowed and remained 6 h for observation. During operations, animals' blood volume was maintained by infusing isotonic NaCl solution. Renal venous blood was collected before the start of ischemia, at 1 h ischemia, 15 min reperfusion following 1 h ischemia, 30 min reperfusion, and every 30 min after that, respectively. Blood samples were transferred to ESR tubes and frozen immediately in liquid nitrogen. ESR measurement were conducted with Bruker ESP 300E spectrometer (X-band) at microwave frequency 9.16 GHz, microwave power 10 mW and modulation amplitude 0.52 mT.

RESULTS

In the venous blood of untreated rats, an ESR signal at g = 2.0 was observed (Fig. 1A), supposed to be semiquinone free radical [3]. In the rats subjected to solely 1 h ischemia a weak signal overlapping on the signal in Fig. 1A was observed (Fig. 1B), then the intensity of this weak signal increased over time (Fig. 1C) and a prominent HbNO signal in triplet hyperfine structure appeared approximately after 2 hours of reperfusion (Fig. 1 D). Then the signal kept the similar form until the end of observation, indicating NO release reached a plateau. Further quantification of blood NO level was determined by estimating pure HbNO spectral [4] and calculating second integrated value of the pure HbNO spectra (Fig. 2).

CONCLUSIONS

Using hemoglobin strapping ESR spectroscopy we were able to carry out real time monitor and quantification of NO level on rat renal I/R model. Results suggest that, after 1 h unilateral renal ischemia, blood NO concentration increased and could be detected by ESR hemoglobin trapping. Blood NO release experienced a gradual increase during reperfusion, and reached a plateau approximately from 2 h reperfusion. This may aid in clarifying the precise role of postischaemic NO and the relevant mechanism.

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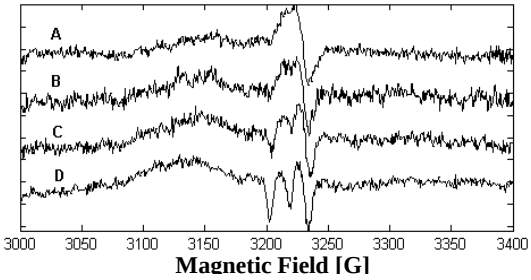

Fig.1. Representative ESR spectra of renal venous blood in rats after 1 h left renal artery occlusion. (A) anesthesia and laparotomy without further ischemia; (B) 1 h ischemia; (C) 1 h ischemia and 1 h reperfusion; (D) 1 h ischemia and 2 h reperfusion.

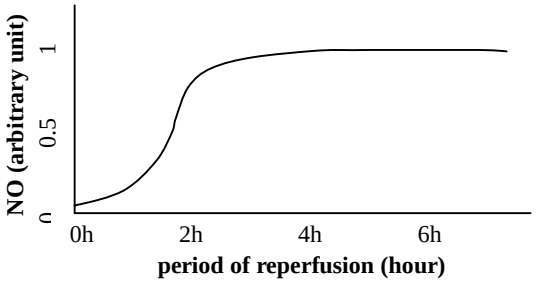

Fig.2. Time-course of renal vein NO level during reperfusion after 1 h renal ischemial