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Corrigendum

# Corrigendum to “Eplerenone promotes alternative activation in human monocyte-derived macrophages” [Pharmacol. Rep. 65 (2013) 226–234]

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An earlier corrigendum has been published but was found to be incorrect. This replaces it. In order to meet high standards of Pharmacological Reports and to improve the clarity of data presentation we wish to perform a corrigendum of methods of western blotting and graphical representation of Fig. 5. In details, western blotting methodology description was improved, proper concentration of eplerenone in Fig. 5 was provided ($10^{-5}$M) and representative blots of Fig. 5 were replaced. On the final steps of figure preparation the graphics technician made an incorrect attachment of blots into Fig. 5. The authors would like to apologize for any inconvenience caused and inform that all made corrections did not affect a scientific merit of the paper.

**Western blot analysis**

Separated proteins were transferred to PVDF membranes (Pall Poland Ltd., Warszawa, Poland) by wet electrotransfer [21]. After the electrotransfer was finished, its proper efficacy as well as the protein load uniformity between the lanes was briefly controlled. To do it, membranes were left to dry for about five minutes and then soaked in a solution containing 20% methanol in water (v/v), which makes the lanes more opaque and thus visible if observed against the light. Membranes were blocked by incubation in blocking buffer (TBST containing 5% non-fat dried milk) for 1 h. Primary antibodies against the mannose receptor, iNOS (C-terminus) (both from Santa Cruz Biotechnology, CA, USA) and arginase I (BD Biosciences, CA, USA) were used at dilutions of 1:300, 1:500 and 1:1000, respectively. Antibodies against β-actin were used at a concentration of 1:3000 (Abcam plc, UK). All antibodies were raised in rabbit. Membranes were incubated with primary antibodies overnight at 4 °C and subsequently incubated for 60 min at RT with anti-rabbit, alkaline phosphatase (AP)-conjugated secondary antibodies (diluted 1:2000 in blocking buffer) (Bio-Rad Laboratories Inc., Hercules, CA, USA). Finally, membranes were washed, and color precipitates were developed directly on the membrane using AP chromogenic substrates (Bio-Rad Laboratories) [20]. The PageRuler Unstained Protein Ladder (Fermentas, Lithuania) served as molecular weight marker. On every gel, the ladder sample occupied the most outer position in the gel and it was cut-out for the separate brilliant-blue staining. Thereafter, the molecular mass of the sample of interest was estimated. The observed band sizes of all analyzed proteins were in concordance with those declared by the antibodies' supplier. The integrated optical density (IOD) of the signals was measured semi-quantitatively using ImageJ software (National Institutes of Health, USA). The expression level of the protein of interest was expressed as relative optical density (ROD). This value was calculated as the ratio of the IOD for the tested protein band to the IOD for β-actin band. The Bioethics Committee of the Medical University of Silesia approved the study. The experiments comply with the current laws of Poland.

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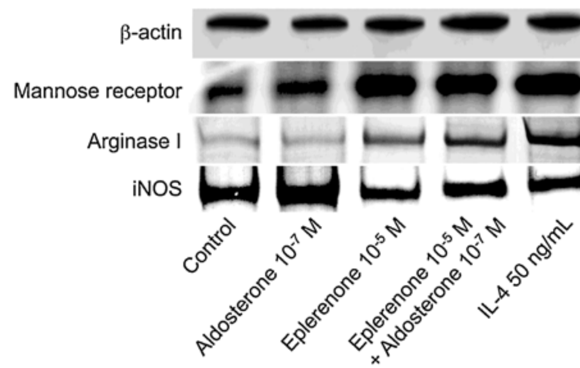


**Fig. 5.** The representative immunoblots of β-actin, mannose receptor, arginase I and iNOS expression patterns. The cultures of human monocytes were treated with medium containing (a) DMSO alone (control), (b) aldosterone ($10^{-7}$ M), (c) eplerenone ($10^{-5}$ M), (d) eplerenone ($10^{-5}$ M, 1-h preincubation) with aldosterone ($10^{-7}$ M), or (e) interleukin-4 (50 ng/ml). β-Actin was measured as a protein loading control. The integrated optical density (IOD) of the signals was measured semi-quantitatively using Image Pro Plus software and expressed as the ratio of the IOD of the tested proteins to the IOD of β-actin. Each experiment was repeated three times, and the relative density values were subjected to statistical analysis.