SciDoc Publishers
Infer, Interpret & Inspire Science

International Journal of Anesthesiology & Research (IJAR)
ISSN 2332-2780

Gene Expression Changes in Response to Vecuronium Bromide in Heart

Research Article

Yao D[#], Lan F[#], Wang T[*]

Department of Anesthesiology, Xuan Wu Hospital, Capital Medical University, Beijing, China.

# These Authors Contributed Equally to this Study and should be Regarded as Co-First Authors.

Abstract

Vecuronium bromide is a non-depolarizing neuromuscular blocking agent and an important muscle relaxant that is cardiovascularly safe and is widely and typically utilized as an adjunct to general anaesthesia. This study sought to examine cellular activity in the heart in response to vecuronium bromide. This study analysed expression profiles in the rat heart after three and five days of exposure to vecuronium bromide. Differentially expressed genes (DEGs) were identified, and functional enrichment analyses were then performed. Three days of exposure to vecuronium bromide altered the expression of 36 genes, many of which are associated with the ubiquitin-proteasome system and steroid response. However, no specific biological process or pathway was enriched. Five days of exposure resulted in 203 DEGs, with the enrichment of genes in biological processes associated with stimulus response and cellular/organism processes and in KEGG pathways associated with the T cell receptor signalling pathway, neuroactive ligand-receptor interaction and the ErbB signalling pathway. We compared expression patterns in the rat heart with those observed in the rat liver, where a different pattern of DEGs involving enrichment in lipid metabolism-associated biological processes and KEGG pathways was detected after five days of vecuronium bromide exposure. Long-term exposure to vecuronium bromidecan lead to stimulus response in the heart but produces a lipid metabolism response in the liver, a metabolic organ.

Keywords: Vecuronium Bromide; Heart; Gene Expression.

Introduction

Vecuronium bromide is a widely used muscle relaxant. It is typically administered via intravenous injection as an adjunct to general anaesthesia to aid endotracheal intubation or for muscle relaxation during surgery. Vecuronium bromide is derived from pancuronium [1] and is categorized as a non-depolarizing neuromuscular blocking agent [2] that competes with acetylcholine for cholinoceptors at the motor end plate to prevent depolarization and thereby exerts muscle-relaxing properties [3]. It has an intermediate duration of effect compared with other non-depolarizing blocking agents [2]. Vecuronium bromide has few side effects. It may cause skeletal muscle weakness, paralysis or respiratory insufficiency. However, it has minimal adverse cardiovascular effects, even in doses far greater than those used clinically [4-7]. Vecuronium bromide is a bisquaternary nitrogen compound in the steroid ester class of organic compounds that features a steroid moiety with a carboxylic acid ester group. The liver is amajor organ in the elimination of vecuronium bromide. In particular, the liver takes up this compound rapidly and excretes both unaltered and metabolized vecuronium [8]. The unchanged drug and its metabolites are eventually removed via renal and faecal elimination.

Recently, the effects of anaesthesia on cells and tissues have been investigated. Gene expression profiling has been used to study the influence of anaesthetics such as isoflurane [9, 10] and sevoflurane [11] in certain organs and tissues. The neuromuscular blocking mechanism of vecuronium has been well-documented [2]. Recent research in rat models has demonstrated that vecuronium-related pharmacodynamic changes in sepsis are associated with the expression of α7- and γ-nicotinic acetylcholine receptor in skeletal muscle [12]. However, molecular mechanisms involved in other vecuronium bromide-related biological activities have rarely been described. No reports have addressed alterations in gene expression profiles following vecuronium bromide exposure. In this investigation, given the availability of gene expression pro-

***Corresponding Author**:
Tianlong Wang,
Department of Anesthesiology, Xuan Wu Hospital, Capital Medical University, Beijing 100053, China.
Tel/Fax: +86-010-83198899
E-mail: w_tl5595@sina.com

Received: July 16, 2017
Accepted: August 08, 2017
Published: August 09, 2017

Citation: Yao D, Lan F, Wang T (2017) Gene Expression Changes in Response to Vecuronium Bromide in Heart. *Int J Anesth Res.* 5(7), 468-472.

files in vecuronium bromide-treated rats, we analysed vecuronium bromide-associated changes in gene expression to reveal relevant biological processes and pathways in the rat heart, which were compared with those found in the rat liver. This study may shed light on the mechanisms underlying the biological activities of different organs/tissues in response to vecuronium bromide.

Materials and Methods

Gene Expression Profiles

Expression profiles for the rat (*Rattus norvegicus*) heart and rat liver (accession numbers GSE59905 and GSE59923, respectively) after exposure to vecuronium bromide were retrieved from the Gene Expression Omnibus (GEO) repository. These data had been profiled using the CodeLink™ UniSet Rat I Bioarray and provided via the DrugMatrix database (https://ntp.niehs.nih.gov/drugmatrix/). The tested rats underwent daily intravenous administration of 0.05 mg/kg of vecuronium bromide in saline. The corresponding control was daily intravenous administration of saline. Three vecuronium bromide-treated and seven control treated heart samples after three days of exposure, six vecuronium bromide-treated and eleven control-treated heart samples after five days of exposure, and three vecuronium bromide-treated and five control-treated liver samples after five days of exposure were assessed. All retrieved expression profile data had already been quantile normalized.

Identification of Differentially Expressed Genes (DEGs)

DEGs were identified using the limma package [13] with a threshold of $p<0.05$, $|\log_2(FC)|>0.5$ (where FC represents fold change) and a false discovery rate (FDR)<0.05. An in-house script was used to convert probe IDs to gene symbols and to delete redundancies. Probes with no matching gene information available in the NCBI were excluded.

Protein-Protein Interaction (PPI) Analysis

PPIs of DEGs were analysed using an online tool for the STRING database (version 10) [14] with a medium confidence threshold (combined score > 0.4). The PPI network was then visualized using Cytoscape [15]. The degree of connectivity of each node was calculated as the number of interactions with surrounding nodes.

Functional Enrichment Analysis

Gene ontology (GO) enrichment analysis for biological processes was conducted online using the PANTHER classification system [16], with a threshold of $p<0.05$. KEGG pathway enrichment analysis was performed using an online tool facilitated by the DAVID database [17], with a threshold of $p<0.05$.

Results

DEGs

A comparison of the test and control groups after three days of vecuronium bromide exposure led to the identification of 36 DEGs in the heart, including 11 up-regulated genes and 25 down-regulated genes (Supplementary Table 1). A comparison of the test and control groups afterfive days of vecuronium bromide exposure resulted in the identification of 203 DEGs in the heart, including 60 up-regulated genes and 143 down-regulated genes (Supplementary Table 2). Eight up-regulated genes and 23 down-regulated genes were detected in the heart after both three and five days of exposure. No significant DEGs in the heart were identified in a comparison of the three-day and five-day test groups. A comparison of the test and control groups after five days of vecuronium bromide exposure led to the identification of 44 DEGs in the liver, including 19 up-regulated genes and 25 down-regulated genes (Supplementary Table 3).

PPI Networks

DEG-related PPI networks were constructed (Figure 1). The PPI network constructed based on the 36 heart DEGs identified after three days of exposure contained only three gene pairs (Figure 1A). The PPI networks for heart and liver DEGs identified after five days of exposure included 145 gene pairs (Figure 1B) and 22 gene pairs (Figure 1C), respectively. Hub genes with a degree of connectivity > 4 in the latter two networks were identified; there were 14 hub genes in the heart and 1 hub gene in the liver (Table 1).

Functional Enrichment Analysis

DEGs in the constructed PPI networks were selected for functional enrichment analysis. The selected DEGs in the heart after fivedays of exposure were enriched in GO biological processes that were mainly associated with stimulus response and cellular/organism processes (Table 2). Genes in five KEGG pathways were also significantly enriched (Table 3). In contrast, selected DEGs in the liver after five days of exposure were enriched in GO biological processes associated with metabolism (Table 2). Genesin two KEGG pathways were also significantly enriched (Table 3). DEGs in the PPI network for the heart after three days of exposure did not exhibit enrichment in any particular GO biological process or KEGG pathway.

Discussion

Vecuronium bromide has minimal adverse cardiovascular effects. Cellular activities in the heart in response to vecuronium bromide have seldom been studied. In this study, we analysed gene expression profiles of the rat heart to investigate relevant cellular activities. The observed pattern was then compared with that of the rat liver, the organ that metabolizes vecuronium bromide.

Vecuronium bromide has exhibited cardiovascular safety. Little is known regarding the biological response in the heart to vecuronium bromide. Research has demonstrated that vecuronium does not affect creatine phosphokinase releasein the heart [18], which can be used to assess heart damage. This study demonstrated the existence of DEGs in the rat heart following vecuronium bromide exposure. DEG patterns for the heart after three and five days of exposure differed. A limited number of DEGs were identified after short-term exposure (three days), whereas longer-term exposure (five days) altered the expression of many more genes. The Pggt1b gene, which encodes the beta subunit of protein geranylgeranyl transferase type I, is one of the most up-regulated genes after three days of vecuronium bromide exposure. Protein

Figure 1. PPI networks of DEGs in response to vecuronium bromide in the heart after three (A) and five days of exposure (B) and in the liver after five days of exposure (C). Nodes represent proteins. Edges represent interactions between proteins. Red nodes represent proteins produced by up-regulated genes. Green nodes represent proteins produced by down-regulated genes. PPI, protein-protein interaction. DEG, differentially expressed gene.

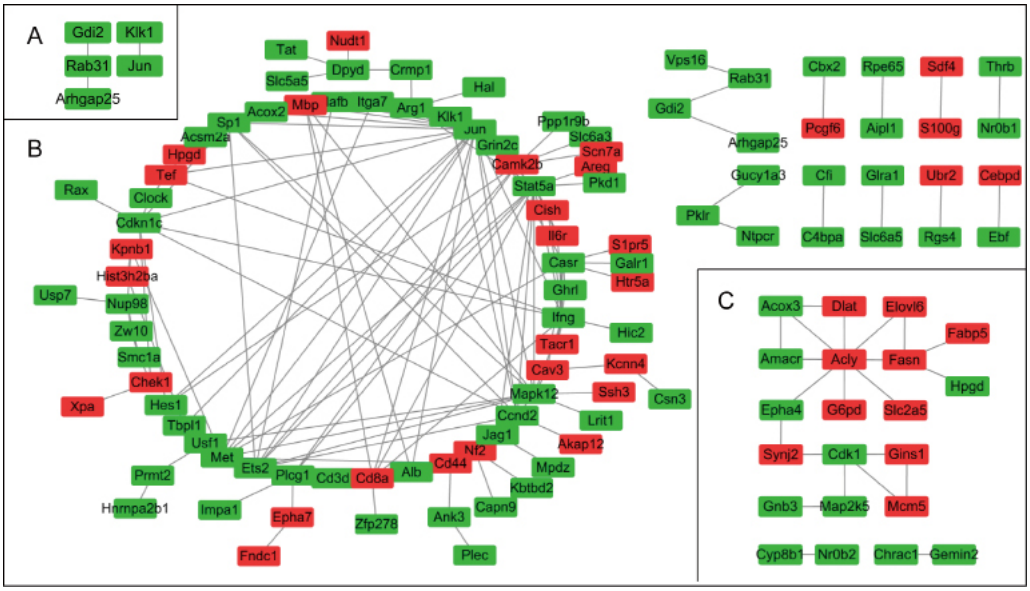

Table 1. Hub genes in PPI networks.

Gene	Up/down	Degree of connectivity
Heart after five days of exposure		
Jun	-	19
Stat5a	-	14
Mapk12	-	12
Ifng	-	10
Met	-	10
Ets2	-	9
Ccnd2	-	9
Cdkn1c	-	8
Casr	-	8
Cd8a	+	7
Camk2b	+	7
Plcg1	-	6
Sp1	-	6
Cd44	+	5
Liver after fivedays of exposure		
Acly	+	8

PPI: protein-protein interaction. +: upregulated; -: downregulated.

geranylgeranyltransferase type I catalyses lipid modification of proteins, and research has demonstrated that Pggt1b is involved in steroid response [19]. The expression of ubiquitin-associated genes, such as *Ubr2, Fbxo2, Fbxw5, Mycbp2, and Usp42*, was also differentially regulated after vecuronium bromide exposure. The up-regulated gene *Ubr2* encodes the ubiquitin protein ligase E3 component n-recognin 2. The E3 ligase plays a vital role in the regulation of the ubiquitin-proteasome system, which is reportedly altered in cardiac disease [20, 21]. However, DEGs after three days of vecuronium bromide exposure were not enriched in any GO biological process or KEGG pathway. In contrast, longer exposure made alterations in gene expression more evident. In addition to genes that already exhibited altered expression, many more DEGs were identified. In particular, the pool of ubiquitin-associated DEGs expanded. Interestingly, the protein Jun encoded by the hub gene Jun can be degraded via the ubiquitin-dependent proteolysis system [22, 23]. Jun is auto-regulated, and its product increases its own transcription. Therefore, the down-regulation of Jun observed here maybe partially associated with ubiquitin-related biological activities. As a transcription factor that responds

Table 2. The Ten Most Significantly Enriched GO Biological Processes.

Term (GO Accession)	DEG Count	p
Heart after fivedays of exposure		
Response to endogenous stimulus (GO:0009719)	32	2.88E-10
Single-organism process (GO:0044699)	87	5.29E-10
Response to stimulus (GO:0050896)	68	7.88E-10
Response to external stimulus (GO:0009605)	33	9.66E-10
Single-organism cellular process (GO:0044763)	80	1.05E-08
Response to organic substance (GO:0010033)	37	1.82E-08
Multicellular organismal process (GO:0032501)	61	2.37E-08
Single-organism developmental process (GO:0044767)	52	2.88E-08
Biological_process (GO:0008150)	96	3.34E-08
Response to organonitrogen compound (GO:0010243)	23	3.77E-08
Liver after fivedays of exposure		
Lipid metabolic process (GO:0006629)	9	4.37E-04
Monocarboxylic acid metabolic process (GO:0032787)	7	9.73E-04
Cellular lipid metabolic process (GO:0044255)	8	9.85E-04
Fatty acid metabolic process (GO:0006631)	6	1.40E-03
Small molecule metabolic process (GO:0044281)	10	1.62E-03
Small molecule biosynthetic process (GO:0044283)	6	3.35E-03
Lipid biosynthetic process (GO:0008610)	6	6.49E-03
Acetyl-CoA metabolic process (GO:0006084)	3	1.52E-02
Single-organism metabolic process (GO:0044710)	13	2.04E-02
Cellular metabolic process (GO:0044237)	17	2.16E-02

DEG: differentially expressed gene.

Table 3. Significantly Enriched KEGG Pathways.

Term (KEGG accession)	DEG count	p
Heart after fivedays of exposure		
T cell receptor signalling pathway (rno04660)	6	2.71E-03
ErbB signalling pathway (rno04012)	5	6.74E-03
Neuroactive ligand-receptor interaction (rno04080)	7	2.82E-02
Haematopoietic cell lineage (rno04640)	4	3.20E-02
Jak-STAT signalling pathway (rno04630)	5	3.49E-02
Liver after fivedays of exposure		
PPAR signalling pathway (rno03320)	3	1.30E-02
Primary bile acid biosynthesis (rno00120)	2	3.70E-02

DEG: differentially expressed gene

to extracellular stimuli, Jun might further regulate the expression of relevant DEGs. In addition, many hub genes are involved in transcription and transduction regulation. Consistently, DEGs after five days of vecuronium bromide exposure were enriched in biological processes associated with stimulus response and cellular/organism processes. With respect to KEGG pathways, DEGs were enriched in the T cell receptor signalling pathway, neuroactive ligand-receptor interaction and the ErbB signalling pathway. Researchers have suggested that the T cell receptor signalling pathway and neuroactive ligand-receptor interaction are often disease-related [24]. Interestingly, a comparison of the gene expression profiles for short- and long-term vecuronium bromide exposure revealed no significant DEGs; this phenomenon may have occurred because the gene expression profile for short-term exposure reflected an intermediate status. It is likely that only long-term vecuronium bromide exposure influences biological activities in the heart. This result confirmed the cardiovascular safety of vecuronium bromide. The effects of affected biological activities require additional investigation.

The liver displayed a different pattern from the heart. Many up-regulated DEGs in the liver are associated with metabolism and

liver disease. The up-regulated Elovl6 gene is a major target for sterol regulatory element-binding proteins. Research has demonstrated that Elovl6 over expression in the liver induces hepatic inflammation and liver injury [25]. In accordance with the known liver function of metabolizing vecuronium bromide, liver DEGs were enriched in biological processes associated with lipid metabolism. Moreover, enrichment was observed in the PPAR signalling pathway and primary bile acid biosynthesis. The PPAR signalling pathway is important for the regulation of lipid metabolism in the liver [26], and bile acids are important signalling molecules that coordinately regulate lipid metabolism [27, 28].

Previous studies have indicated that vecuronium bromide is safe for the heart and liver. This study analysed gene expression profiles following exposure to vecuronium bromide to investigate vecuronium bromide-induced molecular activities in the heart and liver, which may shed light on this agent's mechanisms of action. However, further investigation is required. The understanding of those mechanisms may aid drug development with respect to improving cardiovascular and liver safety.

Conclusion

In the rat heart, DEGs in response to vecuronium bromide were enriched in biological processes associated with stimulus response and cellular/organism processes and in KEGG pathways associated with the T cell receptor signalling pathway, neuroactive ligand-receptor interaction and the ErbB signalling pathway. A different pattern was observed in the rat liver, where DEGs were enriched in metabolism-associated biological processes and KEGG pathways.

Acknowledgements

We thank Shanghai RuiHui Biotech Company, Ltd., for advice regarding bioinformatics analyses and database mining.

References

[1]. Docherty JR, McGrath JC (1980) A comparison of the relaxant and autonomic effects of pancuronium and its monoquaternary derivative Organon NC 45 in the pithed rat [proceedings]. Br J Pharmacol 68(1): 140P-141P.

[2]. Lien CA (2011) Development and potential clinical impairment of ultra-short-acting neuromuscular blocking agents. Br J Anaesth 107 (1): i60-71.

[3]. Bowman WC (2006) Neuromuscular block. Br J Pharmacol 147 (Suppl 1): S277-86.

[4]. Morris RB, Cahalan MK, Miller RD, Wilkinson PL, Quasha AL, et al.,(1983) The cardiovascular effects of vecuronium (ORG NC45) and pancuronium in patients undergoing coronary artery bypass grafting. Anesthesiology 58(5): 438-40.

[5]. Reich DL, Mulier J, Viby-Mogensen J, Konstadt SN, van Aken HK, et al.,(1998) Comparison of the cardiovascular effects of cisatracurium and vecuronium in patients with coronary artery disease. Can J Anaesth 45(8): 794-7.

[6]. Hackett GH, Jantzen JP, Earnshaw G (1989) Cardiovascular effects of vecuronium, atracurium, pancuronium, metocurine and RGH-4201 in dogs. Acta Anaesthesiol Scand 33(4): 298-303.

[7]. Chen BB, Nyhan DP, Blanck TJ (1991) Hemodynamic effects and onset time of increasing doses of vecuronium in patients undergoing myocardial revascularization.J Cardiothorac Vasc Anesth 5(6): 569-73.

[8]. Bencini AF, Mol WE, Scaf AH, Kersten UW, Wolters KT, et al., (1988) Uptake and excretion of vecuronium bromide and pancuronium bromide in the isolated perfused rat liver. Anesthesiology 69(4): 487-92.

[9]. Culley DJ, Yukhananov RY, Xie Z, Gali RR, Tanzi RE, et al.,(2006) Altered hippocampal gene expression 2 days after general anesthesia in rats. Eur J Pharmacol 549(1-3): 71-8.

[10]. Wang H, Jin Y, Dai J (2015) Biological processes and pathway changes in isoflurane-induced anesthesia revealed by bioinformatics analysis of gene expression profiles. J Anesth 29(6): 912-9.

[11]. Sakamoto A, Imai J, Nishikawa A, Honma R, Ito E, et al.,(2005) Influence of inhalation anesthesia assessed by comprehensive gene expression profiling. Gene 356: 39-48.

[12]. Liu L, Min S, Li W, Wei K, Luo J, et al.,(2014) Pharmacodynamic changes with vecuronium in sepsis are associated with expression of alpha7- and gamma-nicotinic acetylcholine receptor in an experimental rat model of neuromyopathy. Br J Anaesth 112(1): 159-68.

[13]. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, et al.,(2015) limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res 43(7): e47.

[14]. Szklarczyk D, Franceschini A, Wyder S, Forslund K, Heller D, et al.,(2015) STRING v10: protein-protein interaction networks, integrated over the tree of life. Nucleic Acids. Res 43(D1): D447-52.

[15]. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, et al., (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. GenomeRes 13(11): 2498-504.

[16]. Mi H, Muruganujan A, Casagrande JT, Thomas PD (2013) Large-scale gene function analysis with the PANTHER classification system. Nat Protoc 8(8): 1551-66.

[17]. Dennis G, Jr., Sherman BT, Hosack DA, Yang J, Gao W, et al.,(2003) DAVID: Database for Annotation, Visualization, and Integrated Discovery. Genome Biol 4(5): P3.

[18]. Wali FA, Makinde V (1991) Effects of pancuronium and vecuronium on creatine phosphokinase in rat isolated heart, liver, kidney and diaphragm. Gen Pharmacol 22(2): 301-4.

[19]. Ellis PJ, Morris TJ, Skinner BM, Sargent CA, Vickers MH, et al., (2014) Thrifty metabolic programming in rats is induced by both maternal undernutrition and postnatal leptin treatment, but masked in the presence of both: implications for models of developmental programming. BMC Genomics (15): 49.

[20]. Mearini G, Schlossarek S, Willis MS, Carrier L (2008) The ubiquitin-proteasome system in cardiac dysfunction. Biochim Biophys Acta 1782(12): 749-63.

[21]. Willis MS, Patterson C (2006) Into the heart: the emerging role of the ubiquitin-proteasome system. J Mol Cell Cardiol 41(4): 567-79.

[22]. Treier M, Staszewski LM, Bohmann D (1994) Ubiquitin-dependent c-Jun degradation in vivo is mediated by the delta domain. Cell 78(5): 787-98.

[23]. Angel P, Hattori K, Smeal T, Karin M (1988) The jun proto-oncogene is positively autoregulated by its product, Jun/AP-1. Cell 55(5): 875-85.

[24]. Gohlke JM, Thomas R, Zhang Y, Rosenstein MC, Davis AP, et al.,(2009) Genetic and environmental pathways to complex diseases. BMC Syst Biol 3:46.

[25]. Matsuzaka T, Atsumi A, Matsumori R, Nie T, Shinozaki H, et al.,(2012) Elovl6 promotes nonalcoholic steatohepatitis. Hepatology 56(6):2199-208.

[26]. Burri L, Thoresen GH, Berge RK (2010) The Role of PPARalpha Activation in Liver and Muscle. PPAR Res 2010 (2010): 1-10.

[27]. Staels B, Fonseca VA (2009) Bile acids and metabolic regulation: mechanisms and clinical responses to bile acid sequestration. Diabetes Care 32(2): S237-45.

[28]. Chiang JY (2009) Bile acids: regulation of synthesis. J Lipid Res 50(10): 1955-66.