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An *In silico* Predictive Analysis of Mepivacaine-GABA Interaction

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SUMMARY. **Gamma-aminobutyric acid (GABA) is an amino acid that is non-proteinogenic in nature, which functions as a predominant neurotransmitter in the central nervous system (CNS) of the human body. Mepivacaine, first introduced in the 1960s, is used as a vasodilator majorly in the fields of dentistry. If the primary functions of both agents may be inhibited by the action of other compounds working in the same manner, it may induce toxicity or stress in the cells. The advertent or inadvertent use of phytochemicals with local anesthetics can result in either synergistic or antagonistic effects in the human body, which is ultimately dependent upon various factors. This elucidation of this type of interaction and the subsequent effects it entailed in the human body, was the main focus of the predictive study. The possible effects of combined administration of Mepivacaine and GABA was investigated by ChemDIS-Mixture (v.5.0, medium confidence score 0.4). The significance level for the analysis was set at 0.05, with Benjamini-Hochberg multiple test correction. The findings of the study elucidated various proteins, signalling pathway, GO, DO, and DOLite terms that are shared between Mepivacaine and GABA when they are taken simultaneously. This study revealed 08 new GO term, 04 signaling pathways, 17 DO terms and 21 DOLite terms associated with their interaction. Personalized treatment with a deeper understanding of known herbal and conventional drugs is the major requirement of the study, which could superlatively prevent any type of antagonistic effects that could take place.**

RESUMEN. El ácido gamma-aminobutírico (GABA) es un aminoácido de naturaleza no proteinogénica, que funciona como un neurotransmisor predominante en el sistema nervioso central (SNC) del cuerpo humano. La mepivacaína, introducida por primera vez en la década de 1960, se utiliza principalmente como vasodilatador en los campos de la odontología. Si las funciones primarias de ambos agentes pueden ser inhibidas por la acción de otros compuestos que actúan de la misma manera, pueden inducir toxicidad o estrés en las células. El uso deliberado o involuntario de fitoquímicos con anestésicos locales puede resultar en efectos sinérgicos o antagónicos en el cuerpo humano, que en última instancia depende de varios factores. Esta elucidación de este tipo de interacción y los efectos posteriores que conlleva en el cuerpo humano, fue el foco principal del estudio predictivo. ChemDIS-Mixture investigó los posibles efectos de la administración combinada de mepivacaína y GABA (v.5.0, puntuación de confianza media de 0,4). El nivel de significación para el análisis se fijó en 0,05, con corrección de prueba múltiple de Benjamini-Hochberg. Los hallazgos del estudio aclararon varias proteínas, vías de señalización, términos GO, DO y DOLite que se comparten entre la mepivacaína y el GABA cuando se toman simultáneamente. Este estudio reveló 08 nuevos términos GO, 04 vías de señalización, 17 términos DO y 21 términos DOLite asociados con su interacción. El tratamiento personalizado con una comprensión más profunda de los medicamentos tradicionales y herbales conocidos es el principal requisito del estudio, que podría prevenir superlativamente cualquier tipo de efecto antagónico que pudiera ocurrir.

KEY WORDS: ChemDIS-Mixture, drug-drug interaction, GABA, local anesthetic, mepivacaine, sodium channels.

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INTRODUCTION

The administration of contraindicating medications of two different natures can ultimately change and alter the pharmacodynamics and pharmacokinetics of either or both of the medications used. More than often, these alterations are not known, which renders it impossible for the side effects to be elucidated in any case. Inducing and inhibiting the hepatic and intestinal drug metabolism enzymes have been indicated in various studies [1-3]. The oral consumption of medicinal herbs may change the gastric and intestinal pH which may lead to the impairment of conventional drug delivery in the human intestine. Such alteration of various factors will subsequently lead to the determination of increased bio-availability of co-administration of various compounds, or vice versa [4]. In the case of co-administration of conventional drugs with herbal medicines, the action of drug metabolizing enzymes could either be induced or inhibited, due to drug transporters being the major targets of herb-drug interactions (HDIs). Therefore, the inhibition or induction of cytochromes may consequently give a possible theory for some HDIs [5,6]. It has been reported that major HDIs entail pharmacokinetic changes which can be measured by the altered activity of cytochrome P450 enzymes [7]. Therefore, the co-administration of herbal medicines with conventional ones lead to the induction or inhibition of the family of cytochrome enzymes [8].

Gamma-aminobutyric acid (GABA) is an amino acid which is non-proteinogenic in nature and is widely reported in various sources such as plants, vertebrates, and bacteria [9]. In vertebrates, it serves a key role as an inhibitory neurotransmitter of the central nervous system (CNS) of the human body, where more than 60 % of the neural synapses are GABA-ergic [10]. Alongwith this widely reported function, GABA-ergic neurotransmitters aid in regulating the brain circuits to modulate anxiety, stress, sleep patterns, memory, mood, and pain [11]. GABA binds to two significant receptors that are post synaptic in nature, the GABA-A and GABA-B receptors, where the former is an ionotropic receptor which elevates the conductivity of chloride (Cl^-) ion influx inside the cell in its presence, consequently leading to hyperpolarization of the cell and the creation of an action potential. The latter serves a significant role as a G-protein coupled receptor which heightens and reduces post-synaptic potassium (K^+) conductivity and pre-synaptic calcium (Ca^{2+}) conductivity, thereby preventing an action potential in the pre-synaptic cell. Since the knowledge of the function of GABA in the treatment of anxiety, sleep disorders, mood enhancement has been widely established, more and more studies have reported its use in the treatment and modulation of pain [12].

Mepivacaine was first used as a vasodilator in the 1960s, with identical anesthetic potential as Lidocaine, but with a milder vasodilating capability [13]. The aim of this study was to predict the possible interaction of mepivacaine with GABA, and the resultant biological pathways, diseases and proteins that are associated positively or negatively to the interactions of both substances. This predictive *in silico* study employed the use of ChemDIS-Mixture, a significant online tool for computational analyses that predicts the potential interactions between two or more substances, thereby identifying the associated molecular pathways, proteins, gene and diseases ontologies that are disrupted or involved due to the interactions of these substances in the human body.

METHODS

Criteria for analysis

ChemDIS-Mixture is an online database, available in the form two versions. Of the two, version 5.0 is more updated and valuable for interaction studies since it can curate more variety of drugs/chemicals from other databases, such as STITCH and PubChem. Similar to STITCH database, three different levels of confidence score are defined at ChemDIS-Mixture as well (low-0.15, medium-0.4, and high-0.7, respectively) (Fig. 1) [14]. The predictive interaction of Mepivacaine with GABA was studied by using a

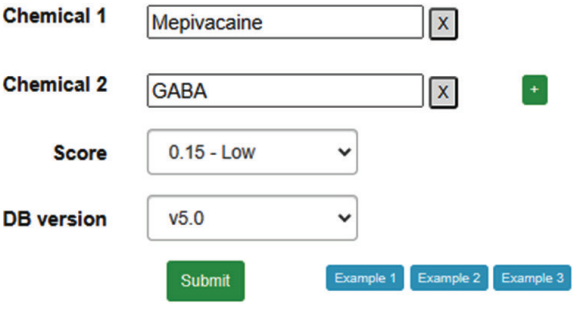

Figure 1. Mepivacaine and GABA were written as the input chemical 1 and chemical 2 in the given slots. ChemDIS-Mixture can study interaction among four chemicals at a time. "Score" represents confidence score, which could be high, medium or low. For this study, medium type score (*i.e.*, 0.4) was utilized.

systematic method, as depicted in Figure 2. The name of each compound was added (Figure 1) into the search bar of the database, followed by adjusting the parameters as a hypergeometric test (Benjamini-Hochberg multiple test correction adjusted to $p < 0.05$) for enrichment analysis of GO, DO, DOLite terms and the associated signaling pathways.

Findings of analysis

ChemDIS-Mixture is an efficient online tool with a vast data of chemicals, drugs and their compounds, which has been sequestered from other databases such as PubChem. Moreover, the tool itself contains a wide variety of target proteins, and their structures curated from other databases. Therefore, the analyses and their outputs performed via this tool are depicted in the form of various target proteins and their associated signaling pathways, GO, DO, DOLite terms which are then represented on Microsoft® Excel® and as mathematical Venn diagrams for depicting the drug-drug interactions (DDI).

RESULTS

A methodical representation of the interaction between Mepivacaine with GABA predicted via ChemDIS-Mixture has been shown in Fig. 2. Venn diagrams are presented in Fig. 3, which were based on the associated proteins, GO, DO, DOLite terms as well as the signaling pathways. The computational analysis also yielded some hyperlinked terms which were then downloaded and tabulated in Microsoft® Excel®.

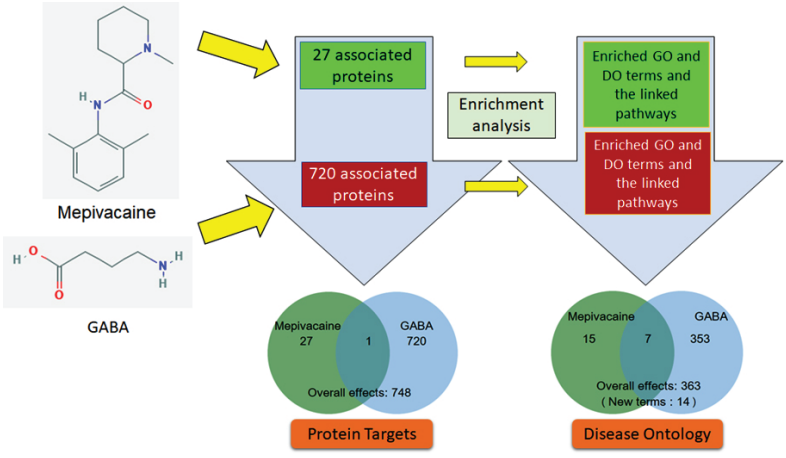

Figure 2. A diagramatic representation of GABA interaction with Mepivacaine studied through ChemDIS-Mixture.

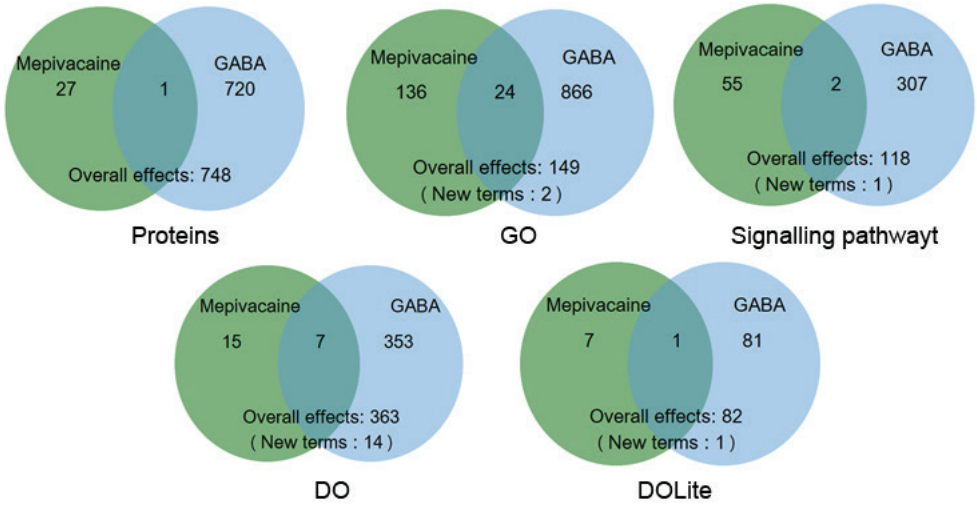

Figure 3. Mepivacaine-GABA interaction in Venn diagrams based on the associated proteins, GO, pathway, and DOLite terms.

Protein analysis

The findings of the study demonstrated 01 protein to beassociated in combination to both agents, while 748 overall effects were found to be generated in the human body through the co-administration of Mepivacaine with GABA (Fig. 3).

GO Term Analysis

The results demonstrated the co-administration of Mepivacaine and GABA to both share 24 GO terms (Table 1; Fig. 3), in comparison to 136 and 866 GO terms of the former and latter agent, respectively. Moreover, 02 new GO terms were, alongwith 149 overall effects were observed from the analysis.

Signaling pathway analysis

The co-administration of Mepivacaine and GABA yielded 55 and 307 SMPs, respectively (Table 2; Fig. 3), while 02 SMPs were found to be similar to both. Overall effects were observed to be 118, with 01 new pathway in the study.

DO Term Analysis

The analysis presented 15 and 353 DO terms for Mepivacaine and GABA, respectively, while the shared DO terms were 7 (Figure 3). Overall effects were reported to be 363, with 14 new DO terms (Table 3).

DOLite term analysis

The results of the study demonstrated that co-administration of Mepivacaine and GABA produced 7 and 81 terms for the former and latter, respectively, whereas 01 DOLite term was shared (Fig. 3). Overall effects produced were 82, of which 01 new effect was observed (Table 4).

DISCUSSION

The predictive analysis of co-administering Mepivacaine along with GABA was investigated int this study. The study findings depicted 02 new GO terms, scaffold protein binding (GOID:0097110), and 4 iron, 4 sulfur cluster binding (GOID:0051539) to be associated with this study. Scaffold proteins are reported to be diverse in their function as they have evolved to perform various functions in cells. Originally idealized to be composed of various interacting motifs or domains, their exact composition can be subject to variation which is largely contingent on the specific pathways that they participate in [15]. Scaffold proteins involved in intra-cellular signaling do not only co-ordinate kinase cascades but also can readily organize other molecules and their signaling processes or cascades [16]. They also tend to regulate interaction at cell signaling junctions, where the generation of pre-formed assemblage allows for the cells to respond efficiently to external stimuli [17]. Moreover, scaffold proteins are significantly associated with the evolution of new biological pathways on the basis of distinct, gene-encoded entities regulating the communication of signaling components. This could thus enable the production of new or recombinant scaffold which could then serve as a common link for pre-existing and newly formed components in new and innovative methods [18,19].

Source	ID	Description	Adj. P1	Adj. P2	Adj. PJoint
MF	GO:0097110	scaffold protein binding	6/ 722	48/ 16309	0.01859
MF	GO:0051539	4 iron, 4 sulfur cluster binding	5/ 722	40/ 16309	0.03066

Table 1. New GO terms obtained as a result of Mepivacaine-GABA co-administration (Unique in overall effect).

Type	ID	Description	Gene Ratio	Bg Ratio	P	Adj. P	Genes
SMPDB	SMP00682	Leucine Stimulation on Insulin Signaling	5/ 113	16/ 1116	0.01709	0.01816	SLC7A5|FRAP1| RHEB2|AKT1|STK14A

Table 2. New signaling pathways obtained as a result of Mepivacaine-GABA co-administration (Unique in overall effect).

DOLite ID	Description	Gene Ratio	Bg Ratio	P	Adj. P	Genes
DOID:1428	endocrine pancreas disease	8/ 492	59/ 8007	0.02683	0.03291	PYY | GHRL | MC4R | STK14A | IL1B
DOID:11260	rabies	8/ 492	60/ 8007	0.02937	0.03542	SCA-1 | PLACK | SNCA | PN2 | PITX2
DOID:5409	lung small cell carcinoma	9/ 492	72/ 8007	0.03135	0.03743	WHIMS | CXCL12 | ASCL1 | MAP2 | RALD
DOID:2237	hepatitis	36/ 492	431/ 8007	0.03568	0.04086	CXCL12 | CXCL11 | CCL21 | CCL20 | CCR2
DOID:1781	thyroid cancer	20/ 492	213/ 8007	0.03783	0.04251	STK14A | PRO2086 | K-REV | SS1R | RAP1A
DOID:3963	thyroid carcinoma	19/ 492	200/ 8007	0.03817	0.04277	STK14A | PRO2086 | K-REV | SS1R | RAP1A
DOID:1100	ovarian disease	8/ 492	64/ 8007	0.04113	0.04510	CXCL12 | PRL | POU5F1 | LF | DRD3
DOID:3347	osteosarcoma	20/ 492	215/ 8007	0.04110	0.04510	WHIMS | CCL5 | STK14A | PRKCG | MTNR1A
DOID:184	bone cancer	22/ 492	243/ 8007	0.04305	0.04638	WHIMS | CCL5 | STK14A | PRKCG | MTNR1A
DOID:2018	hyperinsulinism	7/ 492	54/ 8007	0.04592	0.04831	STK14A | PYY | GHRL | MC4R | BSF-2
DOID:201	connective tissue cancer	31/ 492	370/ 8007	0.04720	0.04844	CCK | CCL5 | STK14A | PRKCG | SCA-1
DOID:4606	bile duct cancer	14/ 492	140/ 8007	0.04830	0.04857	WHIMS | CXCL12 | PRKCA | AKT1 | ERBB2
DOID:4897	bile duct carcinoma	14/ 492	140/ 8007	0.04830	0.04857	WHIMS | CXCL12 | PRKCA | AKT1 | ERBB2
DOID:11612	polycystic ovary syndrome	16/ 492	166/ 8007	0.04847	0.04861	C3 | POMC | CCK | GHRL | MC4R

Table 3. New DO terms obtained as a result of Mepivacaine-GABA co-administration (Unique in overall effect).

DOLite ID	Description	Gene Ratio	Bg Ratio	P	Adj. P	Genes
DOLite:533	Tuberculosis	8/ 289	55/ 4051	0.03932	0.04453	C3 | PARK2 | P2Y1 | AGTR2 | IL1B

Table 4. New DOLites obtained as a result of Mepivacaine-GABA co-administration (Unique in overall effect).

The signaling pathway analysis revealed one new signaling pathway, leucine stimulation on insulin signaling (SMP00682). Elevated levels of plasma of the amino acids such as leucine have been reported to be affiliated with the development of resistance in humans [20]. Therefore, leucine has been suggested to be a major predisposing agent in the development of insulin resistance, which seems to be supported by many recently conducted studies. Comparatively, this notion is observed to be dismissed by some other studies that report no link between leucine and insulin resistance or the improvement in insulin sensitivity [21]. Since the conflict of these studies is dominant, the exact mechanism of leucine and its regulation of insulin resistance is not well elucidated. Nevertheless, it has been demonstrated that leucine infusion can lead to the impairment of glucose uptake in fat (adipose) and muscle (skeletal), which is mediated through the stimulation of secreting insulin [22]. In theory, leucine is able to modulate insulin signaling via the rapamycin (mTOR) and S6K-regulated serine phosphorylation of IRS1-2 [23]. It is important to note

that the presence of leucine in obese animals can lead to an increase in the sensitivity to insulin, as the increase in the branched amino acids via the knock out of rate limiting enzyme is suggested to be proportional to the insulin sensitivity [24]. This elevated sensitivity to insulin by leucine and other branched amino acids could be linked to the reduced consumption of food and heightened energy release from the body [25].

The DO term analysis yielded 14 new terms, known as endocrine pancreas disease (DOID:1428), rabies (DOID:11260), lung small cell carcinoma (DOID:5409), hepatitis (DOID:2237), thyroid cancer (DOID:1781), thyroid carcinoma (DOID:3963), ovarian disease (DOID:1100), osteosarcoma (DOID:3347), bone cancer (DOID:184), hyperinsulinism (DOID:2018), connective tissue cancer (DOID:201), bile duct cancer (DOID:4606), bile duct carcinoma (DOID:4897), and polycystic ovary syndrome (DOID:11612). Hyperinsulinism is a medical condition where insulin secretion is unregulated from cells of the pancreas, resulting in hypoglycemia. It is the most commonly occurring causes of hypoglycemia in neonatal cases [26], with infants facing an elevated risk of permanent brain injury and neurological defects [27]. The disease, in itself, is a broader classification that comprises of various medical disorders that include both (transient and congenital) forms of the disease. Early management of the disease targets the healthy maintenance of normal glycemic levels, which is often achieved through aiding treatments such as glucose infusions, and at later stages, glucagon infusion [28]. Moreover, long-term therapeutic methods comprise of diazoxide as the primary treatment of the disease [29,30].

Limitations of Study

ChemDIS-Mixture is an online tool which aids in the prediction of two compounds which can either be both drugs or phytochemicals and their potential interaction between the two. However, since it is an in silico study, it requires validation in the form of in vitro or in vivo studies, which can affirm the study findings of this analysis and can provide deeper insight into the mechanism of action of both agents.

CONCLUSION

Mepivacaine is a vasodilator which is used in various dental treatments and is used for the management of pain. GABA, on the other hand, serves a major role as an inhibitory neurotransmitter, which also is now reported to stop pain. This study depicted the interaction of both Mepivacaine and GABA when they are taken together, and the potential synergistic or antagonistic effects were also demonstrated. Therefore, it is necessary to carefully monitor the relative case history of the patient when co-prescription of both agents is involves, so as to reduce the chances of any antagonistic effects occurring due to their co-administration in the human body.

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