Beyond single nucleotide polimorphism

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BEYOND SINGLE NUCLEOTIDE POLYMORPHISMS

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ABSTRACT

Similar medications have been known to cause considerable heterogeneity in efficacy and toxicity across human populations. Therefore, individualized, or personalized, therapy has been highlighted as a declared goal of modern medicine. In this paper, we briefly describe the main strategies for dose individualization and then focus our attention on Single Nucleotide Polymorphisms (SNPs), the main source of human genetic and phenotypic variation. This genetic variation was long recognized as the principal genetic contribution to the variability of drug action, but the advent of more powerful molecular technologies has uncovered other abundant DNA variations and changed this perception. It should also be taken in consideration that most drug effects are determined by the interplay of several genes (the genomic approach), rather than candidate gene approaches. Although pharmacogenetics and pharmacogenomics mainly focus on human genetic variations linked to SNPs, we believe that this approach is only a starting point, from which it will be necessary to proceed to a more complex stage of research to better individualize drug therapy.

Key words

Pharmacogenetics; Pharmacogenomics; Pharmacokinetics; Single nucleotide polymorphism

RÉSUMÉ

Il est bien connu qu'un même médicament peut montrer une hétérogénéité dans son efficacité et sa toxicité au sein de l'espèce humaine. Pour cette raison la médecine moderne met de plus en plus l'accent sur une médecine individualisée ou personnalisée. Cet article décrit brièvement les stratégies principales pour une posologie individualisée et focalise ensuite l'attention sur les SNPs (polymorphismes d'une seule paire de base du génome), la source principale de variation génétique et phénotypique humaine. Cette variation génétique a été longtemps reconnue comme la contribution génétique principale à la variabilité de l'action des médicaments. L'arrivée de technologies moléculaires plus performantes a découvert de nombreuses autres variations de l'ADN et a ainsi changé cette perception. Il faut en effet prendre en considération que la plupart des effets des médicaments sont déterminés par l'interaction de plusieurs gènes (l'approche génomique) plutôt que d'un gène unique. Quoique la pharmacogénétique et la pharmacogénomique se focalisent actuellement surtout sur les variations génétiques humaines en relation avec les SNPs, nous croyons que cette approche n'est qu'un point de départ à partir duquel il sera nécessaire d'instaurer des étapes plus complexes de recherche pour aboutir à une médecine mieux individualisée.

Mots clés:

Pharmacogénétique; Pharmacogénomique; Pharmacocinétique; SNP

RESUMEN

Medicamentos similares puede producir una respuesta heterogénea en cuanto a su eficacia y toxicidad. Por lo que una terapéutica individualizada, o personalizada, es considerada como un objetivo específico de la medicina moderna. En este artículo, describimos brevemente las estrategias principales para la individualización de la dosis y posteriormente enfocamos la atención sobre el polimorfismo de un solo nucleótido (PSN), la principal fuente de variación genética y fenotípica humana. Esta variación genética fue extensamente reconocida como la principal contribución genética a la variabilidad de los efectos farmacológicos. Sin embargo, el desarrollo de técnicas moleculares más avanzadas ha descubierto otras variaciones de ADN abundantes y ha cambiado esta perspectiva. También debe de considerarse que la mayoría de los efectos farmacológicos están determinados por la interacción de varios genes por lo que también el trabajo hace un análisis no solo bajo la óptica de algunos genes específicos sino de de una perspectiva genómica. Resumiendo, aunque la farmacogenética y la farmacogenómica enfocan su atención en las variaciones genéticas humanas, principalmente ligadas al PSN, nosotros consideramos que esta perspectiva es sólo el principio desde el cual tendremos necesariamente que movernos a un nivel más complejo de investigación para individualizar la farmacoterapia.

Palabras clave

Farmacogenética; Farmacogenómica; Farmacocinética; Polimorfismo de un solo nucleótido

INTRODUCTION

The rule "the right drug at the right dose at the appropriate time in the right patient" may appear to be a very ambitious goal in drug therapy, but an overview of scientific pharmacological literature over the last 30 years indicates it as one of the main targets in third-millennium medicine.

There are many reasons for the move from the "one drug fits all" approach to personalized medicine: 1) the enormous increase, during the 20th century, in the range of therapies against all major diseases, 2) the increasing life expectancy, 3) drug therapy sometimes fails to be curative, 4) adverse drug reactions are the fifth leading cause of death in the United States [1] [2], 5) powerful new technologies have produced advances in biomedical research.

DOSE INDIVIDUALIZATION

To date, the main strategy for dose individualization is to determine dosage from the drug's pharmacokinetic properties; the classic example of this approach is carboplatin, an analogue of cisplatin, which is used to treat lung and ovarian cancer [3] [4]. Alternatively, initial treatment doses can be used to establish the individual's pharmacokinetic (PK) response to the drug, with subsequent doses being based on this information; this strategy has been used for oral busulfan, an alkylating agent that is used to treat leukemia, which has linear kinetics but high interindividual variability [5] [6] [7] [8] [9].

Another PK-based method for dose individualization is the population pharmacokinetic model. This approach studies the variability of plasma drug concentrations among individuals who receive standard regimens determined from PK data relating to the target patient population. Population pharmacokinetic models aim to account for observed interindividual variation in terms of patient variables called covariates; these include all sources of variability. Population modeling enables the relative importance of covariates to be quantified; Bayesian estimation, which evaluates an individual's data relative to the population pharmacokinetic model, can then be used to estimate patient-specific pharmacokinetic parameters from which to calculate the optimal dose for an individual [10] [11] [12] [13] [14].

Epirubicin, an anthracycline used in breast cancer therapy, and digoxin, used in the treatment of various heart conditions, exemplify how population modeling can lead to dose individualization [13] [15].

However, each of these strategies suffers from some disadvantages, such as subtherapeutic or supratherapeutic dosing during the initial stages and difficulty of adaptation to clinical practice. It has therefore been suggested, following the complete mapping and understanding of all human genes through the Human Genome Project, that pharmacogenetics and pharmacogenomics might have the potential to overcome these drawbacks and to facilitate reaching the goal of optimizing drug therapy [16] [17] [18] [19] [20] [21] [22].

PHARMACOGENETICS AND PHARMACOGENOMICS

The British physician Archibald Garrod was probably the first to realize that certain individuals inherited a predisposition to alcaptonuria or other conditions [23]. In particular, he observed that parental consanguinity was more common than usual among parents of children with alcaptonuria.

However, it was probably William Bateson [24], a biologist who was ahead of his time, who interpreted Garrod's reports as recessive inheritance when he popularized Mendelian genetics in Britain. Bateson discovered genetic linkage and introduced the term "genetics" at some time between 1902 and 1913. With particular foresight, Garrod went on to develop the concept known as the "Chemical Individuality in Man" [24]. He proposed that drugs undergo biotransformation by specific pathways, similar to endogenous substrates. As occurs with inborn errors of metabolism, defects in such pathways could alter drug concentrations and therefore their effects [24].

The concept of familial clustering of unusual xenobiotic responses was reinforced during the 1940s with the observation of a high incidence of hemolysis, on exposure to antimalarial drugs, among individuals with glucose-6-phosphate dehydrogenase deficiency [25]. In the 1950s, Evans et al. identified N-acetylation as a major route of isoniazid elimination [26]. Although individuals varied substantially in terms of the extent to which a single dose of the drug was acetylated, variability between monozygotic twins was found to be small compared with that between dizygotic twins.

This observation laid the groundwork for later studies that have defined the clinical consequences and genetic basis underlying the fast and slow acetylator phenotypes. More generally, the past half century has seen developments towards understanding the molecular basis of drug disposition and action, and of the mechanisms that determine the observed variability in drug action.

The concept of a familial component in drug action thus initiated the field of 'pharmacogenetics' even before the discovery of DNA as the repository of genetic information. An increased understanding of the molecular, cellular, and genetic determinants of drug action elicited an appreciation that variants in many genes might contribute to variability in drug action. The concept of using whole-genome information to predict drug action is one definition of the more recent term, 'pharmacogenomics' [27] [28] [29].

SNP

Whether we are discussing pharmacogenetics - the study of the relationship between individual gene variants and variable drug effects [30] - or pharmacogenomics - the study of the relationship between variants in a large collection of genes, up to the whole genome, and variable drug effects [30] - we are chiefly talking about Single Nucleotide Polymorphisms (SNPs).

An SNP is a change in one nucleotide (base pair) in a DNA sequence. SNPs can be in coding regions (where they may be either synonymous or non-synonymous) or, more commonly, in non-coding regions; they frequently vary with ethnicity [30]. It has been estimated that there are at least 10 million SNPs within the human population [31], averaging one every 300 nucleotides, of the approximately 3 billion nucleotide base pairs that constitute the genome of an individual. It is precisely in these heritable variations among individuals on which the principles of pharmacogenetics and pharmacogenomics are based.

There are many cases in which SNPs have been correlated with significant changes in drug effects [17] [32]. One of the best examples of SNPs relating to the outcome of therapy is the polymorphism of the gene thiopourine S-methyl transferase (TPMT). TPMT is a cytosolic drug-metabolizing enzyme that catalyzes the S-methylation of 6-MP and azathioprine. In their original study, Weinshilboum et al. demonstrated a very clear tri-modal frequency of TPMT activity in red blood cells from 298 unrelated control adults [33]. One in 300 subjects lacked TPMT activity and 11% had intermediate levels. Family studies showed that the frequency distribution was due to inheritance. While phenotypic studies have shown a clear tri-modal distribution, the genetic basis of phenotypic variation has proved more complex.

Seventeen variant TPMT alleles have been identified to date, although 3 variant alleles account for the majority (>95%) of persons with intermediate (1 variant allele) or low (2 variant alleles) TPMT activity [34] [35]. Subsequent clinical studies have demonstrated very clearly that TPMT polymorphism can predict toxicity of 6-MP and consequences of therapy. Children with acute lymphocytic leukemia (ALL) with intermediate or no TPMT activity are at higher risk of myelosuppression when prescribed standard doses of 6-MP [36]. Moreover, a number of studies have shown that TPMT phenotype or genotype influences the effectiveness of therapy, with low TPMT activity being associated with higher levels of cytotoxic 6-thioguanine nucleotides (6-TGN) and reduced relapse [37].

Another example of SNPs influencing therapeutic efficacy involves polymorphism of genes belonging to the superfamily of cytochrome P450 enzymes (CYP) [38]. For example, patients carrying some of the 78 variants of CYP2D6 currently identified (http://www.imm.ki.se/cypalleles) have a greater risk of adverse effects from metoprol, venlafaxine or tricyclic antidepressants [39] [40] [41].
CYP2C19 is important in the metabolism of proton-pump inhibitors (omeprazole, lansoprazole, rabeprazole, and pantoprazole), fluoxetine, sertaline and nelfinavir. Several inactive genetic variants exist, although two (CYP2C19*2 and CYP2C19*3) account for more than 95 percent of cases involving poor metabolism of these drugs [42]. Marked differences in the plasma levels of protein-pump inhibitors occur between genotypes and phenotypes and are reflected in drug-induced changes in gastric pH [43].

CYP2C9 is involved in the hydroxylation of the S-form of the anti-epileptic agent phenytoin and the anticoagulant warfarin. Many CYP2C9 variant alleles have now been reported (http://www.imm.ki.se/cypalleles). Of these, decreased activity has been confirmed in cases with CYP2C9*3, by means of an expression system using COS cells and yeast, and an *in vivo* test on healthy volunteers and patients whose genetic polymorphism was known [44] [45].

For example, oral clearance of (S)-warfarin decreased to below half in subjects with heterozygous polymorphism for CYP2C9*3 (CYP2C9*1/*3) and to below 10% in patients who were homozygous for CYP2C9*3 [46].

BEYOND SNPs

Several pharmacogenetic research studies published in recent decades have demonstrated gene-drug interactions in cases where a SNP has been identified in one or more genes with functional consequences [47]. Nevertheless, many other studies show a poor correlation between SNPs in candidate genes and phenotypes. For instance, Irinotecan is a prodrug that has been widely used to treat advanced cancers and is activated by human carboxylesterase 2. Although the

carboxylesterase 2 gene exhibits several instances of polymorphism, and although an intronic SNP has been found to be associated with reduced carboxylesterse mRNA expression in colorectal tumors, none of the variations in this gene have been found to be associated with protein activity [48] [49]. Furthermore, CYP3A4 is the human enzyme known to be involved in the metabolism of numerous medications. Thus far, no completely inactivating mutations have been discovered in the human CYP3A4 gene, although a common polymorphism in the CYP3A4 promoter has been described [50] [51] [52].

This method is beginning to change as haplotype studies and other approaches appear in the literature [46][30] [53]. An important manuscript in this context evaluated whether the response to inhaled β_2-agonist therapy for asthma was best predicted by individual SNPs or by gene haplotype in the β_2-adrenoceptor gene (ADRB2) [54]. None of the 13 SNPs were significant predictors of response to β_2-agonist therapy. Of the 8,192 haplotypes theoretically possible for (ADRB2), only 5 were commonly observed. Importantly, haplotype analyses did define a patient group with a significantly superior response to β_2-agonist therapy.

A study correlating thiopurine-related adverse drug reactions with TPMT gene polymorphism, one of the best pharmacogenetic examples, noted that 78% of adverse drug reactions were not associated with the TPMT genotype. Researchers are thus moving from SNPs to other human genetic variations, such as small insertions or deletions of repetitive DNA sequences in the promoter region of the TPMT gene, in an attempt to elucidate this association [55] [56].

The idea to move onwards from SNPs to other human genetic variations arises from the advent of genome-scanning technologies that have uncovered an unexpectedly-large number of structural variations in the human genome. These consist of microscopic and submicroscopic variants, including deletions, duplications, insertions, inversions and translocations, which involve segments of DNA that are larger than 1 kb [57].

For example, gene deletions and duplications have been discovered in debrisoquin hydroxylase (CYP2D6), probably the best-characterized genetic polymorphism among the cytochrome P450 enzymes, and concordance between genotype and phenotype has been well established for many drug substrates [58]. However, this monogenic approach, in which a single DNA variant site is associated with a specific alteration, has been criticized in that it fails to consider potential polygenic contributions. For instance, the field of systems biology reflects gene–gene interactions resulting from a particular stimulus that affects a complex circuitry of pathways, ending in a response by the cell or organism [59]. Gene–gene sensing, or gene–gene warfare within the genome, has been called molecular drive or meiotic drive [60]. Gene conversion can lead to one gene repairing, or altering the expression of, its neighbor gene [47]. Gene silencing can occur through several different mechanisms, including DNA hypermethylation and RNA interference [61].

Genomic imprinting also results from DNA methylation [47]. Nutrition and dietary supplementation have been shown to affect epigenetic gene regulation in man [62]. Extensive transmission distortion can lead to unequal genetic sharing among relatives [47]. Several studies have suggested that more than 70% of all human multi-exon genes are alternatively spliced [47]. Exonic splicing enhancers can be disabled by a synonymous single-nucleotide polymorphism, regarded by many as unimportant compared to a non-synonymous single-nucleotide polymorphism [47]. Stochastic events (random noise during transcription and other cellular processes) can also markedly affect gene expression [47]. Another area of active inquiry is the transcriptional regulation of normal proteins, which can be highly variable because of allelic variants in regions of DNA that regulate expression [63] [64].

Variation in the function or expression of genes encoding various factors, such as AhR (arylhydrocarbon receptor), PPAR (peroxisome proliferator activated receptor), PXR (pregnane X receptor) and CAR (constitutive androstane receptor), that control the transcription of genes encoding drug-metobolizing enzymes and transporters, could also contribute to variable drug action [65] [66] [67] [68] [69]. Finally, transcriptional mutagenesis results in mutated mRNA and, therefore, in mutated protein, although the mutation does not exist in the DNA [47].

CONCLUSIONS

Although pharmacogenetics and pharmacogenomics focus attention on human genetic variation mainly linked to SNPs, we have shown in outline that this approach is only a starting point, from which it will be necessary to proceed to a more complex stage of research. We believe, however, that SNPs comprise a useful step in individualizing therapy, although we are less optimistic than some [70]. The chief role of SNPs, in our view, is as one covariate in population pharmacokinetic models [71] [72], with the goal of preventing subtherapeutic or supratherapeutic dosage (during the initial stages) in particular therapeutic groups [73] [74]. They may also help to avoid high-risk subjects developing severe adverse drug reactions [35]. In

summary, it is clear that no single approach is likely to identify, in all individuals, the contribution made by all genes and gene products responsible for a particular drug response.

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CONFLICT OF INTERESTS/DISCLAIMERS

RC is member of the Editorial Board of the journal.

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