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PHARMACOEPIDEMIOLOGY AND PRESCRIPTION

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Risk of drug-induced agranulocytosis: the case of calcium dobesilate

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Abstract *Background*: In the last 20 years, some cases of agranulocytosis associated with calcium dobesilate consumption in Spain have been reported. A high risk of dobesilate-associated agranulocytosis (121 cases per million per year) calculated using both a case-control and a case-population strategy has been published. However few spontaneous reports have been noted in the same period of time. No explanation exists for this disagreement.
Methods: Estimated incidence rates of agranulocytosis in the IAAAS study and the calculated risk of dobesilate-associated agranulocytosis were used as background risks in a Poisson-based methodology, to calculate the number of coincidental reports of agranulocytosis among patients treated with dobesilate. The influence of treatment duration, notification rate and population characteristics were calculated.
Results: During the period 1978–2000, a total of 23 cases would have taken place if the background risk of agranulocytosis were 4.7 per million per year (IAAAS's risk); however, only 9 spontaneous cases of agranulocytosis associated to dobesilate were noted. A simulation showed that with notification rates equal to or higher than 17%, it was not possible to exclude that the 9 cases were false-positives. With notification rates equal or inferior to 16%, it would be unlikely that cases of agranulocytosis were noted in this population with a risk of 4.7 per million per year; therefore, it is necessary to assume a higher agranulocytosis risk. More than 1 case per year could be a false-positive if the background risk of agranulocytosis is 9.5 per million per year, this being the appropriate risk for a population of patients older than 60 years. The duration of treatment beyond 30 days increases the probability of a random coincidence of the intake of drug and an agranulocytosis event.
Conclusions: The disagreement between calculated dobesilate-associated agranulocytosis risk and the number of noted spontaneous reports may be explained by at least three different factors: under-reporting, duration of treatment and age of patients. It is possible, with the methodology presented, to estimate the influence of these factors to avoid confusion with possible false-positive cases and then to design the correct prospective trial that can provide the true agranulocytosis risk.

Keywords Calcium dobesilate · Agranulocytosis · Case-control study

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Introduction

Blood dyscrasias account for only a small percentage of total cases of adverse drug reactions (ADRs) reported to authorities; however, they are responsible for a large proportion of cases associated with a fatal outcome [1]. A population case-control study, the International Agranulocytosis and Aplastic Anaemia Study (IAAAS) provided an overall estimated incidence rate of agranulocytosis in the general population of 4.7 per million per year and an overall estimated incidence rate attributed to drugs of 3.1 per million per year [2]. It was noticed that the incidence and mortality increased dramatically with age [1].

In 1992, results were published on the comparison of risk estimates for agranulocytosis, associated with sulphasalazine and trimethoprim–sulphamethoxazole

therapy, in the Swedish Drug Monitoring System (''spontaneous'' reports, sales and prescription information) and in the IAAAS study [3]. Data from both sources provided a similar estimate of the risk of developing agranulocytosis associated with the two drugs. These results agree with the widespread opinion that serious (potentially fatal), rare and/or bizarre spontaneous reports of drug-related events are less affected by high levels of under-reporting [4].

Calcium dobesilate is used in the treatment of diabetic retinopathy and chronic venous insufficiency. In the last 20 years some cases of agranulocytosis associated with calcium dobesilate consumption in Spain have been reported. The risk of dobesilate-associated agranulocytosis calculated using both a case-control and a case-population strategy has been published [5]. The disagreement between the agranulocytosis risk calculated in the case-control study (121 cases per million users and per year) and the few spontaneous reports noted during the same period of time ($n = 9$) contrasts with risk estimates for sulphasalazine and trimethoprim–sulphamethoxazole. In this study, we have analysed different factors that might explain this disagreement, i.e. agranulocytosis background risks, increases of risk associated with dobesilate consumption and efficiency to identify new cases of dobesilate-associated agranulocytosis, avoiding possible false-positives.

Materials and methods

An overall estimated incidence rate of agranulocytosis of 4.7 per million per year, an estimated incidence rate attributed to drugs of 3.1 per million per year and a mortality rate of 9% were obtained from the IAAAS study [1]. Similarly, age-estimated incidence and mortality rates were obtained from the IAAAS study [1]: (1) 2–24 years: 1.1 per million per year (mortality 5%); (2) 25–59 years: 2.7 per million per year (mortality 9%); and (3) >60 years: 9.5 per million per year (mortality 12%).

The incidence of agranulocytosis in Spain among users of calcium dobesilate (121 cases per million per year), calculated using a case-control strategy with a case-population approach consumption data, was obtained from the study of Ibañez et al. [5].

Spontaneous reports of agranulocytosis associated with calcium dobesilate consumption in Spain from 1978 to 2000 were identified using a Medline search and post-marketing surveillance data provided by the manufacturer of calcium dobesilate. Consumption of calcium dobesilate during this time period was expressed as defined daily doses (DDDs) per inhabitant and per year; as the DDD of calcium dobesilate has not been established, we used a DDD of 1 g to treat venous insufficiency and diabetic retinopathy [5]. Consumption data for the period 1978–2000 were available from the manufacturer.

The number of patients treated in a year (Nt) was calculated using the equation $Nt = DDDyr/Dt$, where DDDyr is the calculated number of DDD in a year and Dt is the average duration of treatment (days) with dobesilate. If Pp is the background risk, the expected number of coincidental associations among treated patients during a year is $Nt \times Pp$. If R is the assumed percentage of cases that are reported, the exact number of coincidental reports is: $Mp = Nt \times Pp \times R \times Dt/365$ [6]. In the context of post-marketing surveillance Nt is expected to be large and Mp small. Thus, the probability P(k) of receiving k coincidental reports can be calculated using the Poisson distribution [7]. For a given value of Mp and an alpha error of 5%, it is then possible to calculate the critical value of K (Kc) for which $P(\geq K)$ becomes smaller than 0.05 (the maximum number of reports one can accept as coincidental) [6]. A sensitivity analysis was conducted to estimate Kc considering Dt ranging from 30 days to 365 days, different background risks (Pp) and a variety of R values, from 0.5% to 100%. Likewise, $P(\geq K)$ was calculated with different Dt, Pp and R.

Results

From 1978 to 2000, a total of 5.16×10^{11} mg calcium dobesilate (tablets of 250 mg and 500 mg) was sold in Spain, a country with a mean of 37 million inhabitants during this period. Sales were not uniformly distributed throughout the period of time considered; hence, 1.88×10^{11} mg calcium dobesilate was sold from 1978 to 1989 (1.57×10^{7} DDD per year), 2.62×10^{11} mg from 1990 to 1998 (2.91×10^{7} DDD per year) and 6.66×10^{10} mg from 1999 to 2000 (3.33×10^{7} DDD per year). In patients with venous insufficiency, the observed mean length of treatment with 1 g/day calcium dobesilate was 30 days/year; in patients with diabetic retinopathy, the treatment period extended to 300 days/year (data obtained from the manufacturer market studies).

A total of 17,215,339 treatments or patients exposed to calcium dobesilate in the period 1978–2000, equivalent to 748,493 patients or treatments per year ($Nt = 748{,}493$), was calculated assuming a mean Dt of 30 days and 5.16×10^{11} mg dobesilate sold. Supposing a background risk of 4.7 per million per year, the maximum number of reports that one can accept as coincidental or false-positives (Kc) was 23 (1 report/year). For a risk of 9.5 per million per year, Kc was 46 (2 reports/year). When longer Dt was considered, Nt diminished while the factor Dt/365 increased in equal proportions; so, under these conditions, Kc was not modified. During the period 1978–2000, a total of 9 spontaneous reports of agranulocytosis in patients treated with calcium dobesilate was notified to the Spanish Drug Surveillance Program, a number well below that of possible false-positives.

This reduced number of reports notified to the Spanish Drug Surveillance Program can be explained as a consequence of under-reporting. Influence of under-reporting was analysed using different simulations. Assuming a risk of agranulocytosis of 4.7 cases per million per year, the notification of one report in a year in a population of 748,493 patients was explained by $R \geq 17\%$. This value of 17% was a detection limit [Rp(1)] above which it was probable to identify one or more reports of agranulocytosis. If $R < 17\%$, the probability that a report were notified in a year was smaller than 0.05; then, if 1 or more reports were identified, a risk of agranulocytosis higher than the one initially assumed must be considered. When the risk was 9.5 per million per year, Rp(1) was 8%. With higher risks, Rp(1) was close to 0 because of the expected high number of reports. It was surprising that under these circumstances no reports were noted. Therefore, an R value [Rp(0)], above which a probability smaller than 0.05 that no reports were notified in a year, was calculated. When

R≥Rp(0) and no reports of agranulocytosis were detected in a year, a new risk of agranulocytosis smaller than that initially considered must be assumed.

In Fig. 1 the distributions of the probability that K cases appear, assuming a risk of agranulocytosis of 121 per million per year and R of 20, 50 and 100%, have been plotted. For this background risk and R≥40%, the probability that no reports of agranulocytosis were notified in 1 year in the population studied (Nt = 748,493) was below 0.05.

From 1978 to 2000, 9 spontaneous reports of dobesilate-associated agranulocytosis were notified. Assuming that R = 40%, a total of 22–23 cases of agranulocytosis would have happened during the period, equivalent to a risk of 1.3 per million per year. Simultaneously, an R≥17% (R≥8% for older than 60 years) permits to explain the identification of 1 report per year as a coincident report or false-positive, for a background agranulocytosis risk of 4.7 per million per year.

All these simulations are influenced by the number of patients or treatments (Nt) calculated using the sales of the drug and Dt. Nt could change if the drug is not sold uniformly, neither during the time period considered nor in different geographical areas. Although we did not have the annual sales of dobesilate nor their geographical distribution, we knew the milligrams sold during the periods 1978–1989, 1990–1998 and 1999–2000. Therefore, we repeated, for the three periods, the same analysis made previously. The result of this analysis is shown in Table 1. The quantity of dobesilate sold increased with time; so did the number of patients treated in a year (Nt). During the period 1978–1989, Nt was 522,639 and Rp(1) was 26% for a background risk of 4.7 per million per year. During this same period, Rp(0)≥57% when the risk of agranulocytosis was 121 per million per year. Table 1 shows that, for this same risk, the Rp(0) required during the two following periods, in which the sale of the product increased, were lower than 30%. During the period 1995–2000, 3 of the 9 spontaneously notified reports were published [8, 9, 10].

The 12 cases of agranulocytosis by dobesilate described in the study of Ibañez et al. [5] and the 9 spontaneous reports (Table 2 and Table 3) were from patients older than 60 years; furthermore, those patients were being treated with one or more drugs in addition to

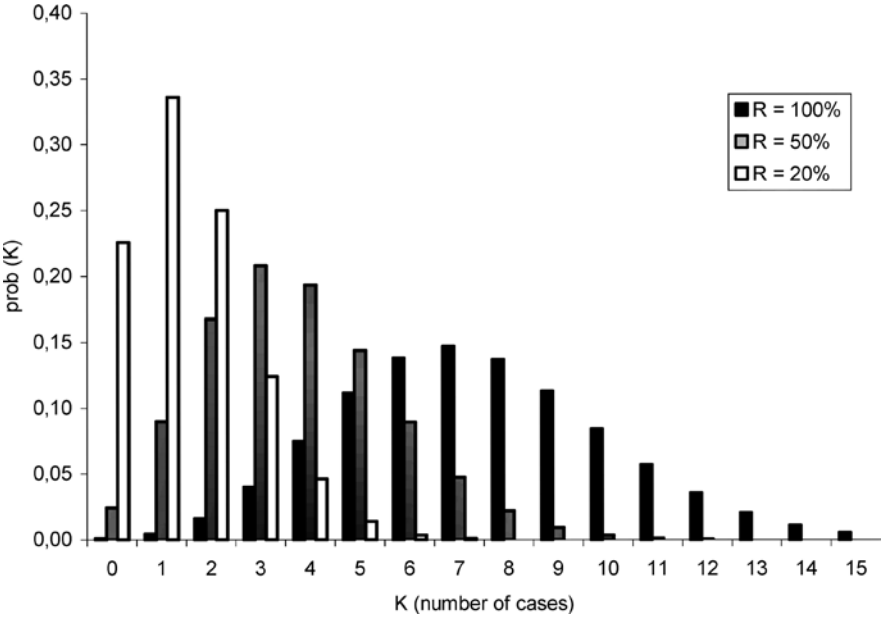

Fig. 1 Probability of appearance of K cases of agranulocytosis per year supposing a risk of 121 cases per million per year and three different rates of spontaneous notification R: 20, 50 and 100%

Table 1 Analysis of the effect of the notification rate and the number of patients treated, assuming two different risks of agranulocytosis. *Nt* number of patients treated in a year, *No. of cases* number of cases spontaneously notified in the period, *Kc* maximum number of cases one can accept as coincidental in the period (false-positives), *Mp* exact number of reports of coincidental associations, *Kc* and *Mp* were calculated assuming a notification rate (R) = 100%, *Rp(1)* notification rate above which it was likely to identify one or more reports of agranulocytosis that take place as a function of the background risk considered, *Rp(0)* notification rate above which the probability that in ayear no reports were noted was smaller than 0.05, *Dt* 30 days

Period	No. of years	mg sold	Nt	No. of cases	Risk = $4.7/10^6$/year			Risk = $121/10^6$/year Rp(0)
					Kc	Mp	Rp(1)	
1978–1989	12	1.88×10^{11}	522,639	2	12	0.20	26	57
1990–1998	9	2.62×10^{11}	969,444	7	18	0.37	14	31
1999–2000	2	6.66×10^{10}	1,109,339	0	4	0.43	13	27

Table 2 Description of the nine spontaneous reports of agranulocytosis. *Latency period* period of time between the beginning of in take of dobesilate and the appearance of agranulocytosis

Case number	Latency period	Date of agranulocytosis	Age/sex	Number of concomitant treatments
1	6 years	22/10/85	91/Female	5
2	Several years	7/1986	72/Female	5
3	9 months	1994	70/Female	8 (positive re-challenge)
4	6 months	10/11/95	68/Female	4
5	2 months	09/10/96	64/Male	4
6	3 months	04/05/96	75/Male	3
7	2.5 months	09/06/96	73/Male	4
8	Long term	25/05/97	93/Female	3
9	21 days	15/07/98	75/Female	5

Table 3 Description of the 12 casesof agranulocytosis in the case-control trial [9]. *Latency period* period of time between the beginning of in take of dobesilate and the appearance of agranulocytosis

Case number	Latency period	Date of agranulocytosis	Age/sex	Number of concomitant treatments
1	6 years	22/10/85	91/Female	5
2	1 month	29/07/86	77/Female	5
3	2 years	27/05/88	66/Female	4
4	1 month	05/07/88	66/Female	3 (Same patient as the previous one)
5	1 year	30/07/90	75/Female	2
6	55 days	08/10/90	76/Female	3 (Same patient as the previous one)
7	23 days	26/06/94	63/Female	4
8	31 days	15/03/95	61/Female	3
9	82 days	10/05/96	75/Male	2
10	83 days	14/06/96	73/Male	4
11	31 days	04/04/97	63/Female	1
12	7 days	16/09/97	60/Female	5

dobesilate. A significant proportion of these patients took dobesilate during prolonged periods of time (years) before suffering the agranulocytosis event. One of the spontaneous reports was of a new agranulocytosis episode after re-challenge to dobesilate, while two of the cases in the case-control study suffered agranulocytosis after re-challenge to dobesilate and concomitant drugs.

An evaluation of the influence of age, a factor associated with a high risk of agranulocytosis, was carried out. The maximum number of coincidental cases of agranulocytosis that would appear in 1 year in a population of 748,493 patients, assuming different number of patients older than 60 years, was calculated (assuming $R = 100\%$ and background risks of 4.7 per million per year for patients younger than 60 years and 9.5 per million per year for patients older than 60 years). When the number of patients older than 60 years was smaller than 25%, the maximum number of cases was 1. This number was doubled when the percentage of patients older than 60 years was greater than 25%.

The estimation of a risk using consumption data of a drug depends on the duration of treatment. The probability that agranulocytosis and drug exposure coincide increases with the time of drug exposure. To evaluate the impact of the duration of treatment, a maximum number of coincident cases of agranulocytosis in 1 year (Kc), assuming risks of 4.7 and 9.5 per million per year and different Nt values, were calculated. The results of this analysis are shown in Fig. 2. For each risk and Nt, Kc increased with the duration of treatment. Even with a small number of patients, the increment of Kc was observed with treatments prolonged more than 1 month, in those patients aged 60 years and more than 2 months in the general population. In patients older than 60 years, the number of cases increased when the duration of treatment exceeded 6–9 months.

Discussion and conclusions

The systems of spontaneous notification and case-control trials are good tools to detect problems related to ADRs. However, they do not permit the calculation of the absolute value of a risk. The most important epidemiological study carried out until now, the IAAAS [2], has provided a measure of the risk of agranulocytosis in the general population after excluding tumours, immunodeficiencies and chemotherapy, radiotherapy or immunosuppressive therapy. This study demonstrated that the risk is not uniform, varying according to the age of the population exposed and the geographical area [1].

It is possible to estimate the number of patients treated with a drug in a geographical area and during a period of time, using the sales of the drug, the DDD and the number of inhabitants. In any case, this approach is weak because of well-known confusion factors; for instance, the sales and consumption of a drug are different and the doses used can differ from the DDD. However, there are other factors, usually not considered, that are key to interpret the resultant risk.

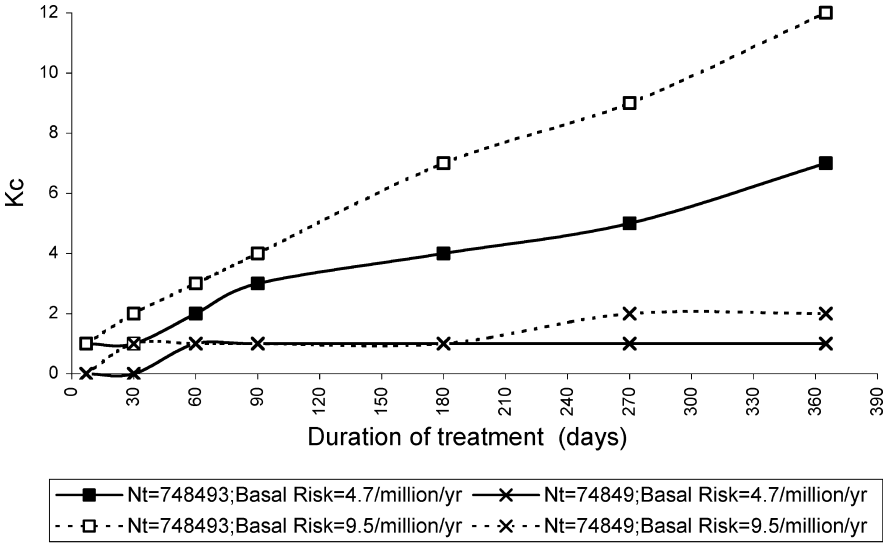

Fig. 2 Estimation of the maximum number of coincidental cases of agranulocytosis (Kc, ordinate), that appear supposing risks of 4.7 and 9.5 cases per million per year, as a function of the different number of patients treated (Nt) and the different durations of treatment (Dt) with calcium dobesilate (abscissa, 7, 30, 60, 90, 180, 270 and 365 days). A notification rate (R) of 100% was considered in all the analyses

One of these factors is the limitation to detect new drug-induced agranulocytosis. The problem of dobesilate-induced agranulocytosis is an illustrative example of the influence of this factor, because cases of agranulocytosis have been identified by two systems with different detection capabilities, i.e. spontaneous notification and a case-control trial. During the period 1978–2000, a total of 23 cases (1 case per year) would have taken place in a population of 748,493 patients treated during a year, assuming a DDD of 1 g, a period treatment of 30 days and a risk of agranulocytosis of 4.7 per million per year. During this period, 9 spontaneous cases of agranulocytosis associated with dobesilate were noted. A simulation using different notification rates showed that with notification rates equal to or higher than 17%, it was not possible to exclude that the 9 cases were false-positives. With notification rates equal or inferior to 16%, it would be unlikely that cases of agranulocytosis were noted in this population with a risk of 4.7 per million per year; therefore, it is necessary to assume a higher agranulocytosis risk. Then, if we know the true notification rate of agranulocytosis of a system, we can calculate the threshold or limit of the number of notified cases above which we must consider a higher risk.

This threshold did not stay constant with time since, as shown in Table 1, the annual sales of the drug increased, but the number of notified cases did not. The appearance of a case of agranulocytosis per year must be explained by an increment of the background risk if notification rates were inferior to 26% during 1978–1989, 14% during 1990–1998 and 13% in the years 1999 and 2000. This change in notification rate could be indicative of deterioration of the Spanish Drug Surveillance Program or of different agranulocytosis risks, among dobesilate-treated patients.

The IAAAS study showed that the population risk of agranulocytosis is influenced by the patient's age. The 9 spontaneous reports of agranulocytosis noted from 1978 to 2000 were patients older than 60 years (Table 2), which is consistent with the indications for use of calcium dobesilate. If we assume that the whole population of patients treated with dobesilate were older than 60 years of age, notification rates below 8% allow us to suspect an increment of the risk when one or two reports of agranulocytosis in a year were notified. Considering 25% or more of the population older than 60 years, up to two cases of agranulocytosis in a year could be ascribed to false-positive.

In the study of Ibañez et al. [5], an estimate of the risk of agranulocytosis associated with dobesilate was estimated using all consecutive cases identified through active case surveillance and the number of patient years calculated from sales data. Applying to our analysis the risk estimated in this study, 121 per million per year, the probability that no cases were noted in 1 year, as it happens in 16 of the 23 years considered, was smaller than 0.05; except when we consider under-reporting rates below 57% during 1978–1989, 31% during 1990–1998 and 27% in the years 1999 and 2000. Therefore, we will have to accept that the increase in sales of the drug, and presumably of the number of patients treated, has been accompanied by a progressive decrease in the notification rate. At the same time, the notification rate should be very low, lower than 13–14%, to reject that the appearance of one case in a year was a false-positive. Even more than 1 case per year could be a false-positive if the risk of agranulocytosis is 9.5 per million per year, the appropriate risk for a population of patients older than 60 years.

The term patient years does not take individual exposure duration into consideration and therefore can underestimate the number of exposed patients. Moreover, the duration of treatment influences the number of false-positive cases detected. The magnitude of this effect is evidenced in Fig. 2, in a simulation in which two populations with a different number of patients treated were considered. When the population of exposed patients is high enough, the effect of the duration of

treatment is evident. However, even in small populations the duration of treatment beyond 30 days increases the probability of a random coincidence of the intake of drug and an agranulocytosis event. This is not an insignificant fact if we consider that most cases of agranulocytosis associated with dobesilate (Table 2) took the drug during long periods.

We have presented a methodology useful to investigate the limits of the risk associated with drug-induced agranulocytosis, calculated using spontaneous reports or case-control trials and drug consumption data. With this approach, it is possible to estimate the influence of under-reporting and the confusing influence of false-positive cases. In the example of dobesilate, we cannot conclude that this drug had not been the cause of some or many of the agranulocytosis cases reported, but we can calculate the notification rates that should be given to accept some risks and to estimate the efficiency of a notification system. The noise of any drug-associated event, of a given detection system, depends on factors such as the patient's age and the duration of drug treatment. Both factors can change the perception of a risk because they can influence the number of false-positives detected. From the dobesilate example, it is evident that the two agranulocytosis identification systems used have been useful to alert of a possible higher risk. However, the hypothesis that a certain risk exists must be accepted only after discarding that detected cases can be explained by smaller risks.

Therefore, the question that we should ask when we analyse a risk estimated using spontaneous reports and/or case-control trials information is whether the calculated risk overcomes in a significant way the background risk of agranulocytosis in the general population. To answer this question, it is necessary to analyse the influence of several factors that can make us confuse coincident false-positive cases with true drug-associated cases. When calculated risks using spontaneous reports and case-control trials are very different, the analysis proposed here may be very useful to estimate if the differences can be influenced or not by confusion factors. When an influence of confusion factors is suspected, only a prospective study with an appropriate follow-up of patients treated with the drug can provide us the true agranulocytosis risk associated with the drug.

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