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British Journal of Anaesthesia, 126 (6): 1097−1102 (2021)
doi: 10.1016/j.bja.2021.03.009
Advance Access Publication Date: 20 April 2021

Applying the adverse outcome pathway concept to questions in anaesthetic neurotoxicity

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Keywords: adverse outcome pathway; $GABA_A$ receptor; general anaesthesia; neurotoxicity; NMDA receptor

Adverse outcome pathways (AOPs) aim to improve research synthesis through structured, multilevel integration of basic science and data from human trials.[1] The AOP approach is endorsed by the Organisation for Economic Co-operation and Development (OECD)[1] and used by toxicologists to aid evidence synthesis in the face of an ever-increasing volume of highly specialised biomedical data.

The AOP concept gained acceptance in regulatory toxicology after a landmark report from the US National Academy of Sciences in 2007.[2] That report recognised that existing practices were insufficient for effective and timely risk assessment of chemicals because of the rapidly expanding chemical industry.[2–4] The central tenet of the proposed strategy to improve risk assessments was to develop *toxicity pathways*—a process of delineating the sequence of key events at different biological levels (molecular, cellular, tissue, and organ) resulting from chemical perturbation of a biological process or system.[2] The AOP concept evolved from this, broadening the approach to include effects at the level of an organism or population.[5,6]

So far, AOPs have been developed to address endpoints relevant to regulation and safety of chemicals. However, the approach has far wider application than within toxicology. The systematic organisation and appraisal of biomedical data at the core of AOP development echo methods of literature analysis that are already central to clinical research, but do not encompass mechanistic data. Adoption of the AOP framework as a complement to systematic review and meta-analysis would significantly aid integration of preclinical and clinical data sets.

There are particular advantages in applying an agnostic science-based strategy, such as AOPs, in anaesthetic research, specifically in paediatric neurotoxicity. In 2017, conclusions drawn about the safety of anaesthesia in children less than 3 yr old instigated regulatory involvement from the US Food and Drug Administration.[7] Ultimately, a warning was issued, highlighting concerns that the developing brain could be adversely affected by prolonged exposure to anaesthetic drugs. This has since generated contention amongst experts and international discussion about how to advance research in this complex field.[8]

For a subject area where expert opinion is staunchly divided, the opportunity to display available evidence in a single integrated platform is appealing. Using the AOP framework, knowledge of the current distribution of evidence would be more accessible, enabling transparent data analysis and identification of critical knowledge gaps. It is hoped that this would facilitate harmonisation of expert opinion, aid future trial design, and in time may also be used to inform regulatory decision-making.

Structure of the AOP framework

An AOP provides a clearly accessible, multiscale overview of the known molecular and cellular events linking a biological stressor to an adverse outcome in an individual or population. An example of an AOP structure is shown in Fig. 1.

The AOP approach was formulated in 2012 by the OECD Extended Advisory Group on Molecular Screening and Toxicogenomics, and is the most readily usable component of the broader AOP Knowledge-Base initiative.[9] To develop an AOP, relevant literature is used to identify crucial biological events leading to an observed adverse effect. Specific terminology for each event is stipulated,[10] beginning with a *molecular initiating event* (MIE), which describes the primary interaction of a stressor with a biological system at a receptor or molecular level. Subsequent events are termed *key events* (KEs), and the final event is the *adverse outcome* (AO). The MIE and AO are referred to as anchors of the AOP.

A KE refers to a specific biological object, such as an organelle, enzyme, or tissue type, which undergoes a measurable process or change in a certain direction that results in impaired or inhibited functioning of the system (e.g. 'ionotropic gamma-aminobutyric acid [GABA] receptor chloride channel conductance, reduced'.[11] As KEs are used to describe a situation, where normal biological function is compromised beyond the capacity for homeostatic mechanisms to compensate, a KE is sometimes described as a motif of biological failure.[1] The title of a KE is formulated according to this structure. Measurement of the KE using simple laboratory or other appropriate techniques should be apparent from the title of the KE and briefly described. It is intuitive that an assay measuring intracellular chloride concentration would be required to identify occurrence of the KE in the preceding example. In addition, reference ranges for expected levels of enzyme activity in the relevant biological compartment would be helpful in defining whether an activity was indeed decreased by the exposure.

A structured record of evidence supporting a KE is documented in a KE description.[10] Every KE must be part of the causal pathway between exposure and AO rather than simply an effect of a chemical exposure; this is termed *essentiality*. The essentiality of each KE is substantiated by the evidence contained in the KE description and is evaluated according to the following three domains:

(i) Current understanding of the basic biological function or role served by the KE.
(ii) A description of established methods for measurement and or detection of the KE.
(iii) Evidence of the specificity of the KE to a certain life stage, species, tissue type, or sex, called the *domain of applicability*.

The relationship between each pair of adjacent KEs is also described in a structured format, termed a KE relationship (KER) description.[10] This process examines the empirical evidence demonstrating that at sufficient concentration and duration of exposure, cell defence mechanisms and adaptation processes will be overcome, triggering the next KE. There is clear guidance on the configuration of a KER description, which should encompass the following domains:

(i) Biological plausibility of the KER, which may be well-established knowledge.
(ii) Experimental evidence that development of the earlier KE leads to the later KE.
(iii) Summary of incongruences or inconsistencies in the experimental evidence in the second domain.

Data permitting, it is possible to quantify a KER in terms of dose—response magnitude and whether there is a threshold for a given adverse effect.

Finally, an assessment of confidence in the overall AOP should be undertaken. Predefined criteria for grading the essentiality of each KE as high, moderate, or low according to the presence or absence of direct, indirect, or contradictory data are provided within OECD guidance.[10] Similarly, a list of defining questions for grading confidence in the biological

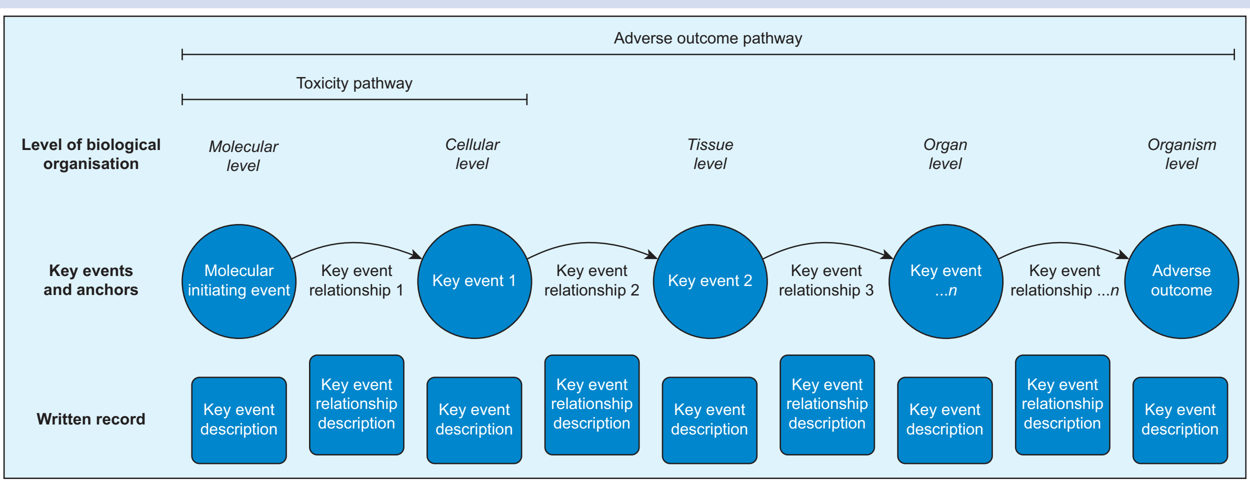

Fig 1. Basic structure of an adverse outcome pathway. The molecular initiating event is the first anchor. This is an interaction with a chemical, which is sufficient to perturb molecular-level homeostatic mechanisms and cause disruption of normal biological function at progressive steps in the pathway (i.e. moving up the chain from cellular to tissue to organ level). This can result in the second anchor, an adverse outcome occurring in an organism or population. A toxicity pathway constitutes the early stages in the adverse outcome pathway, encompassing molecular- and cellular-level perturbations, and focused on the induction of toxicity in a biological system.[23]

plausibility and empirical support for each KER is stipulated. Together with the modified Bradford Hill criteria,[10] these gradings are used to generate an overall weight of evidence conclusion, which is an accepted approach to evidence appraisal in toxicology.[12]

Important considerations for AOP development

Adverse outcome pathways should be developed in accordance with a number of underlying assumptions.[1] The first of these is that the process is intentionally reductionist, simplifying complex biological processes to focus only on specific events that disrupt normal function when perturbed beyond a certain threshold.[15] Secondly, given the intended modular structure of AOPs and the fact that there are frequently several KEs at each level of biological organisation, it should be possible for KEs to be integrated into AOP networks. It is widely acknowledged that biological processes do not operate in such a discrete manner as implied by individual linear AOPs. Over time, however, commitment to constructing AOPs in a modular fashion will contribute to an interface, which better resembles systems biology.[1,10,13] Thirdly, AOPs should be chemically agnostic. This means a given AOP should be generalisable to the effect of any stressor demonstrated to trigger the MIE, rather than a description of the effects of one chemical.[1]

Application of the AOP approach to anaesthetic neurotoxicity

Anaesthetic neurotoxicity provides a good model for demonstrating the use of AOPs in clinical research. Currently, mechanistic understanding of adverse neurodevelopmental effects attributable to general anaesthesia in early life is incomplete.[14,15] To construct an AOP network for this problem, available literature should be collated using a systematic search strategy, and stratified, for example, according to anaesthetic drug exposure or a specific developmental window. Careful data extraction of the experimental methods and endpoints reported in each article are then used to identify key themes and indicate how the literature is clustered across different levels of biological organisation. In time, this builds a profile for the exposure of interest and its mechanistic link to the AO. A flow chart demonstrating the approach to developing an AOP is shown in Fig. 2. For example, collated studies investigating exposure to known N-methyl-D-aspartate (NMDA) receptor antagonists might collectively demonstrate clusters of evidence for NMDA receptor antagonism, impaired synaptogenesis, and worse performance in tests of learning and memory.[16] These three clusters constitute plausible KEs in the AOP *neurodevelopmental toxicity attributable to NMDA receptor antagonism*. The result is a multilevel literature review presented as a dynamic infographic. The next stage in AOP development is to formulate KER descriptions. This involves a second round of systematic retrieval and evaluation of evidence, this time directed towards the link between KEs (i.e. a modified systematic review of evidence that NMDA receptor antagonism causes impaired synaptogenesis). Identified studies are analysed qualitatively (e.g. for risk of bias) and quantitatively (tabulating exposure dose, duration, and interval for all studies). Ideally, this would be done using validated tools appropriate to the type of study in question; however, in practice, this is difficult to achieve, and appropriate tools for every study type are not currently available.

Existing AOPs have examined some molecular-level interactions pertinent to anaesthetic drug exposure, including stimulation of ionotropic GABA type A receptors[11] and NMDA receptor antagonism.[17] At the time of writing, the online repository for AOPs contains more than 50 KEs and KERs relating to GABA-mediated neurotransmission and NMDA receptor action.[18] Details of these AOPs are publicly available online through the AOP wiki.[18] Specifically, there is significant work examining the relationship between NMDA receptor antagonism during brain growth, and impaired learning and memory in childhood.[17] It is likely that this work could contribute to the paediatric neurotoxicity knowledge base.

Advantages and disadvantages of the AOP approach

Broadly, advantages of the AOP concept include the ease with which information can be stored, accessed, and examined once the basic structure has been built. Although AOP development is a labour-intensive and time-consuming process, the result is a ‘living’ review.[10] In other words, additional findings that support or refute existing evidence for a KE or KER can be added as the data are identified, meaning AOPs evolve to reflect the scientific progress on a given subject and are not reliant on the completeness of a single initial search strategy. In addition, incorporating contradicting data or studies negating a relationship between KEs aids avoidance of publication bias.

An AOP presents known information in a format that is easily accessible to researchers from any scientific discipline. This facilitates close scrutiny of evidence by experts in different fields, ultimately improving the accuracy and understanding of the subject area in question.

Undertaking research for the protection of human health requires a reliable means for determining the adequacy of the evidence base to address a specific regulatory endpoint or health effect. Therefore, in order for AOPs to be used in the context of regulatory decision-making, ultimately there should be a procedure for addressing questions, such as how reliably an AOP predicts toxic endpoints, what level of uncertainty can be tolerated in this specific context, and what the level of evidence is.[10] In each case, these questions vary, depending on the intended application of the AOP. Meeting the requirements of the chemical risk assessment field poses different challenges to those faced in healthcare. As an example, there might be less emphasis on determining an acceptable margin of uncertainty when implementing the AOP concept for hypothesis generation compared with setting regulatory exposure limits. However, if the approach were used to set thresholds for a biomarker to rule a diagnosis in or out, a greater certainty would be required.

In relation to paediatric neurotoxicity, expert opinion acknowledges the pragmatic difficulties and high costs of further observational or interventional human trials aiming to characterise AOs in children.[19,20] Such difficulties are attributable to numerous confounding factors and the complex interplay of social factors, school attendance, influence of pain, and quantitative analysis of learning and memory, amongst many others. A recent systematic review examining the heterogeneity of neurocognitive outcomes in studies investigating effects of anaesthesia in children concluded that consistency in these studies is lacking.[21] In the face of such complex issues, it may be justifiable to reflect on the existing wealth of data on

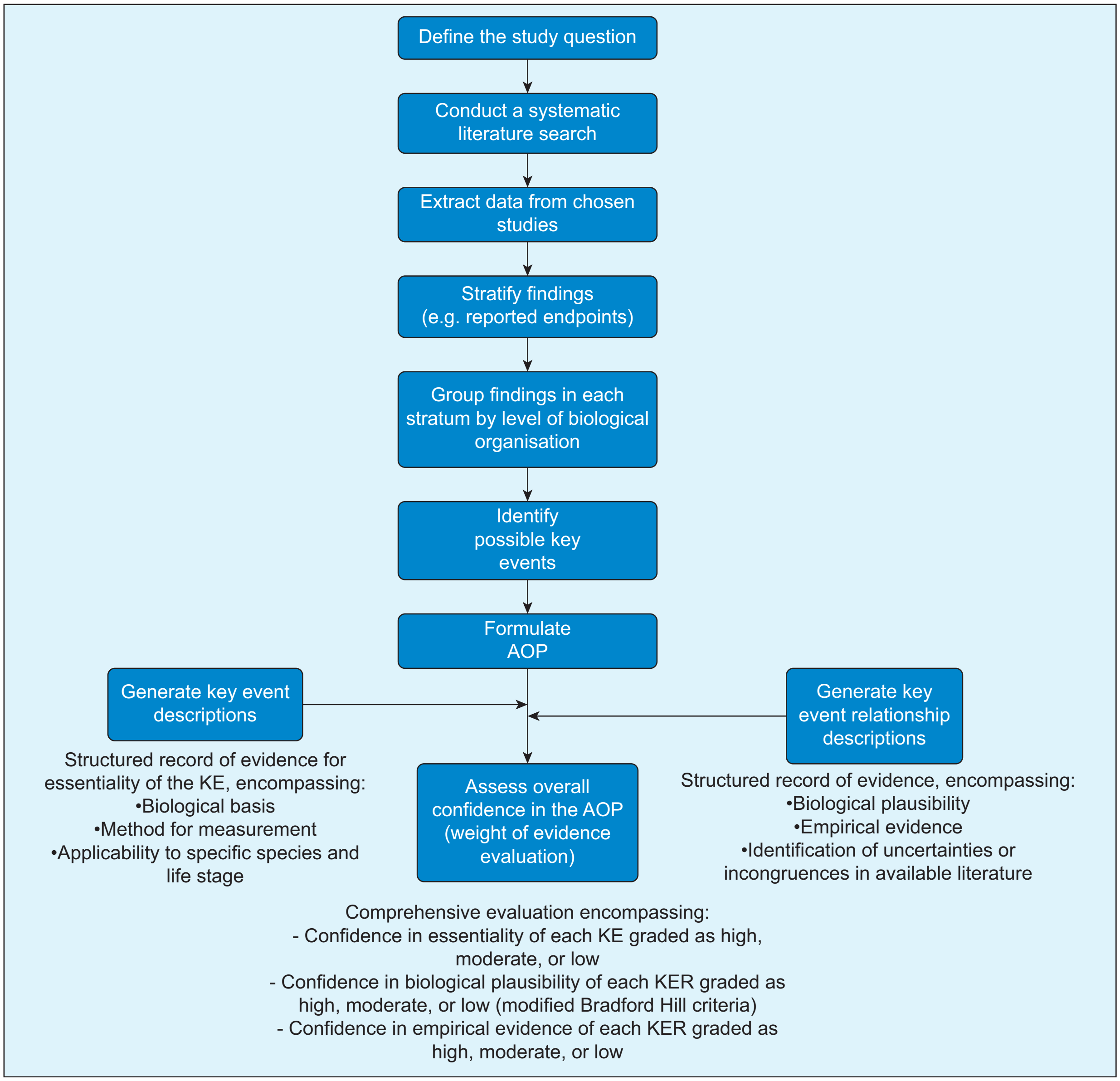

Fig 2. Flow chart indicating the basic process for developing an adverse outcome pathway (AOP), including aspects of pathway evaluation. KE, key event; KER, key event relationship.

this subject, pause further experimentation or clinical trials, and explore mechanistic aspects *via* the AOP framework. Ideally, this would direct focus towards elements of, for example, learning or behaviour, which are more likely to be functionally impaired,[22] or direct future trials to a specifically vulnerable group in terms of the timing or duration of anaesthetic exposure.

Evidence appraisal in toxicology and clinical research

There is currently no formal procedure for evidence appraisal in the AOP development process. This is in stark contrast to the heavy emphasis on systematic review methodology in clinical research, which has only recently been adopted into chemical risk assessment and toxicology.[23] Although this is a potential weakness of the AOP approach, there are pragmatic difficulties in defining one method for critically appraising a range of studies inclusive of *in vitro*, *in silico*, *in vivo*, or human data, which may all be relevant to a single KE. So far, the approach relies very much on expert judgement.

Certainly, methods for evaluation of risk of bias and reliability for *in vitro* and *in vivo* studies are less established than in clinical research, and this is an area of controversy particularly in chemical risk assessment.[24] This may present an opportunity to advance methods for evidence appraisal across multiple study types, and promote harmonisation of study evaluation in different scientific disciplines.

Another essential component of systematic review is conducting a structured and comprehensive literature search. During the identification of KEs, a review of the existing literature is required to accrue information about the plausible mechanisms and intermediate steps leading to the final adverse effect. As such, it is intuitive that incorporation of a predefined method for identifying relevant studies would be useful. However, owing to the possibility to add new data to an existing AOP framework over time (which is desirable), a comprehensive search is not essential for a first iteration.

Finally, to progress the development and implementation of AOPs in clinical research, possible sources of funding should be considered. Given the broad applicability of the AOP approach and the nature of the work as secondary research, it is logical that organisations involved in method development, critical appraisal, and evidence integration would be best suited to support and advance AOP projects. Example organisations may include Cochrane or Grading of Recommendations, Assessment, Development and Evaluations[25]; however, at present, any such funding remains to be sought.

Future perspectives

There is a growing perception that the traditional classification of disease is likely to change as mechanistic science advances, resulting in a new disease taxonomy.[26] An example of this is PRECISESADS, an ongoing multicentre, non-randomised, cross-sectional study across 18 European centres.[27] The aim of this project is to use machine learning and bioinformatics to analyse biological samples from individuals affected by systemic autoimmune diseases. When complete, the analysis will encompass genetic and omics data (epigenomic, transcriptomic, and proteomic, amongst others) with a view to reclassifying the cohort according to the underlying mechanics of the disease process, rather than clinical presentation.[27] The AOP concept strongly supports a grassroots approach to molecular disease classification, and there are advantages to adopting this in anaesthesia.

Anaesthetic research faces complex questions, including which individuals will suffer from postoperative delirium or cognitive decline, and how these risks can be mitigated through our practice. In these cases, an established AOP framework could provide a road map of up-to-date multilevel evidence to guide decision-making and support a molecular-level profile for each phenomenon. With over 200 AOPs under development, it seems likely this approach will be increasingly implemented in the groundwork for future biomedical research. However, the potential value of AOPs in relation to clinical questions is yet to be realised.

Authors' contributions

Paper conception/writing: JW, TGH.
Revision/approval of final paper: TGH, TJAC.

Acknowledgements

The authors would like to thank Anna Bal-Price from the European Commission, Joint Research Centre, Ispra, Italy, for her support in the preparation of this paper.

Declarations of interest

The authors declare that they have no conflicts of interest.

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